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Genetic Analysis of Drought Tolerance in Rice (Oryza sativa L.)

Uttam Bhattarai
ubhatt3@lsu.edu

GENETIC ANALYSIS OF DROUGHT TOLERANCE IN RICE (*Oryza sativa* L.)

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Uttam Bhattarai
B.S. Tribhuvan University, Nepal, 2010
M.S. Assam Agricultural University, India, 2013
M.S. Louisiana State University, Baton Rouge, USA, 2017
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Abstract

Drought is a major challenge in rice production system worldwide. We conducted a phenotypic screening of the USA rice genotypes for drought tolerance and assessed genetic diversity using SSR markers. Identification of QTLs for drought tolerance during both vegetative and reproductive stage was done using genotyping by sequencing (GBS) based saturated SNP linkage map. The rice genotypes Jes, Leah, Roy J, Jazzman, and Madison were found to be drought tolerant. Population structure analysis grouped the USA rice genotypes into California, Louisiana, and Arkansas types. Marker-trait association showed that markers RM570 and RM351 were significantly associated with grain yield, spikelet fertility, and harvest index with 7% of the phenotypic variance. RM302 and RM461 were significantly associated with shoot dry weight with 9% of the phenotypic variance. Fourteen additive QTLs were identified for root length, shoot length, fresh root mass, fresh shoot mass, number of tillers, dry root mass, dry shoot mass, and root-shoot ratio. A majority of the drought responsive QTLs were located on chromosome 1. The expression of QTLs varied under stress and irrigated conditions. Shoot length QTLs *qSL1.38* and *qSL1.11* were congruent to dry shoot mass QTL *qDSM1.38* and dry root mass QTL *qDRM1.11*, respectively. Analysis of genes present within QTL intervals revealed many potential candidate genes such as laccase, Calvin cycle protein, serine threonine protein kinase, heat shock protein, and WRKY protein. In the reproductive stage drought screening, 21 QTLs were discovered for days to flowering, plant height, leaf rolling score, plant dry matter content, spikelet fertility, grain yield, yield index, and harvest index. A major QTL for plant height *qPH1.38* was identified in a narrow confidence interval on chromosome 1. The QTLs, *qDTF3.01* and *qPH1.38,* overlapped with the previously identified QTL *qDTY1.1* and *Hd9,* respectively. A large-effect QTL *qLRS1.37* was identified close to the *sd1* locus on chromosome 1. A grain yield QTL *qGY1.42* located on chromosome 1 contained only 4

candidate genes. There was no overlapping of QTLs for the root traits and the yield attributes. The important candidate genes present within the large effect drought tolerance QTL regions are MYB transcription factors, no apical meristem protein (NAC), potassium channel protein, nuclear matrix protein1, and chlorophyll A-B binding protein. The drought tolerant US rice genotypes identified in the genetic diversity analysis will be valuable for breeding programs whereas the candidate genes and the QTL information will set the foundation for application of marker-assisted pyramiding approach to improve drought tolerance in rice.

Chapter 1. Introduction

1.1. Rice: Importance and production

Rice (*Oryza Sativa* L.) is an important cereal crop in the world both in terms of production and consumption. It is the staple food for almost half of the world's population. Almost 90% of rice is produced in Asia (USDA 2018a). The global production of rice in 2017 was 725 million metric tons (USDA 2018b). China is the major producer of rice followed by India. Rice production in USA is concentrated in six major states, *viz.* California, Arkansas, Louisiana, Texas, Mississippi, and Missouri. The USA is ranked sixth among the rice exporting countries in the world with an annual export of 3.3 million metric tons in 2017. Among the US states, Arkansas produces the largest amount of rice followed by California and Louisiana (Statista 2018). The total area under rice cultivation in the USA in 2017 was 996 thousand hectares with a total production of 9 million metric tons (USDA 2018b).

1.2. Rice water requirements

Rice is the biggest user of irrigation water in the world accounting for 34-43% of the total world's irrigation water or 24-30% of the entire world's fresh water resources. Rice is grown continuously flooded from transplanting until 7-10 days before harvest (Rice knowledge bank, IRRI, http://www.knowledgebank.irri.org/step-by-step-production/growth/water-management). The water requirement in rice varies depending upon the rice growing ecosystem. Rice is grown in both lowland and upland ecosystems. The water requirement of rice in lowland ecosystem is quite high. On an average, the water requirement of rice during its entire growth period to produce 1kg of rice is 1300-1500 mm, which is equal to 1432 liters of water. More than 90% of rice in the world is grown under lowland rice ecosystem (Halwart and Gupta 2004). The water requirement depends on the soil type of the rice growing area. Rice is usually grown in clayey

soils and the water requirement in heavy clay soil is 400 mm compared to 2000 mm in coarse-textured soil (Rice knowledge bank, IRRI, http://www.knowledgebank.irri.org/step-by-step-production/growth/water-management). The rise in temperature is expected to increase the water requirement for rice cultivation by 2-5% in 2046. Climate change is also expected to decrease the precipitation, which may affect the rainfed rice growing ecosystem in the world (Wang et al. 2012).

1.3. What is drought?

In general, every drought definition is associated with low availability of water. Wilhite and Glantz (1985) classified drought into four major categories: meteorological, hydrological, agricultural, and socio-economic drought. Agricultural drought is of the major concern in crop growth and production and is defined as the insufficiency of water for crop growth. The level of water requirement in crops differs depending on the type of plant and its growth stage. Drought can be classified into three major categories based on the timing of its occurrence in lowland rice cultivation systems (Chang et al. 1979). Vegetative drought occurs at the seedling stage and is less harmful to the plants because they have optimum time to recover even after drought. Intermittent drought occurs between the rainfall events. It affects the plant‘s vegetative development. Terminal drought occurs during the flowering stage resulting in drastic reduction in grain yield. Plant breeding programs for drought tolerance are designed for a specific environmental condition called target environment (Fischer et al. 2003). Multiple target environments can be defined for a crop. The target environment for a lowland rice breeding program may depend on the rainfall pattern, soil type, frequency, and severity of the drought events.

1.4. Drought responses in rice

Rice exhibits a large reduction in grain yield under drought. Rice plant displays various mechanisms to cope with the drought stress. Drought avoidance is a mechanism in which the plant tries to maintain its osmotic level either by developing a deeper root system or by reducing the plant water use. Delay in flowering is another strategy adapted by plants to escape from the drought environment. Drought tolerance is the ability of the plant to minimize reduction in grain yield even after exposure to drought conditions (Kamoshita et al. 2008). Several phenotypic and physiological adaptive mechanisms protect rice plants under drought stress.

Higher leaf rolling, reduction in spikelet fertility, and reduction in grain yield are some of the major phenotypic changes in rice plants under drought stress (Bhattarai and Subudhi 2018a). Other physiological responses of plants under drought include reduction in leaf water content, increased canopy temperature, reduction in chlorophyll content, and increased proline accumulation (Shukla et al. 2012). Seedling stage drought screening can be done to assess drought tolerance in rice (Kato et al. 2008). However, it may not be the primary indication of the plant's drought tolerance ability for sustainable yield. Most of drought tolerance studies conducted at reproductive stage are based on plant height, days to flowering, and yield (Ghimire et al. 2012; Palanog et al. 2014; Prince et al. 2015). Roots traits are important attributes that help rice plant to withstand drought. Drought tolerant plants tend to increase root length under drought stress (Bhattarai and Subudhi 2018b). Root length, root volume, root mass, and root angle are some important traits for consideration in drought tolerance screening.

Identification of traits that can distinguish drought tolerant varieties from susceptible ones is necessary for any successful breeding program. Drought tolerance traits in rice have been classified as primary, secondary, integrated, phenological, and plant type traits (Kamoshita et al.

2008). Primary traits can be constitutive traits (root depth, branching angle) or induced traits (hard pan penetration, osmotic adjustment). Plant type traits (plant height, number of tillers) and phonological traits (days to flowering) are the characteristics of the plant and are highly heritable. Secondary traits (relative leaf water content, leaf rolling score, canopy temperature) are the combined effect of the primary traits and have influence on integrated traits (harvest index, spikelet fertility, grain yield).

1.5. Genetic diversity in rice

Genetic diversity study among the available germplasms is necessary before starting any crop improvement program. It helps to assess variability among the genotypes and identify suitable donors. Rice is a highly diverse plant species with various genome compositions (AA, BB, CC, DD, EE, FF, GG, HH, JJ, KK, and LL). The genus *Oryza* is comprised of 27 species and only two (*O. sativa and O. glaberrima*) are cultivated species (GRisP 2013). The classification of *Oryza sativa* into two sub-groups, *indica* and *japonica*, has been widely accepted. There are approximately 140,000 rice genotypes in the world. The International Rice Research Institute (IRRI) has preserved 100,000 rice genotypes from all around the world (FAO 2003). The USA rice genotypes are less diverse. Most of the southern USA rice varieties were found to be derived from 22 plant introductions and the rice varieties from California were found to be derived from 23 introductions in early 1900s (Dilday 1990).

Various techniques have been used to study genetic diversity in rice. Principal component analysis and cluster analysis have been extensively used to group the genotypes (Das et al. 2013). Molecular markers like SSR and SNPs are widely used to uncover the small genetic variations among the individuals. The population structure analysis, a model-based approach, is

broadly used as a tool to understand the genetic variability (Das et al. 2013). Analysis of molecular variance, discriminant analysis, and principal coordinate analysis are also used.

1.6. QTLs for root traits under drought stress

The drought tolerance ability in rice depends on the root system. A drought tolerant genotype tends to increase root length and number of lateral roots under drought stress (Zheng et al. 2003; Uga et al. 2013). Various QTLs for root length, root thickness, and root penetration ability have been detected and incorporated into rice varieties to enhance drought tolerance (Steele et al. 2006). Besides these, some large effect QTLs were detected for root penetration index and root pulling force on chromosome 4 (Zhang et al. 2001), root penetration index on chromosome 3 and 10 (Ali et al. 2000). Zheng et al. (2003) identified 18 QTLs for seminal root length, adventitious root number, lateral root length, and lateral root number. Incorporation of these QTLs into the elite germplasm could be useful to increase the drought tolerance in rice.

1.7. QTLs for yield and agronomic traits under drought stress

Enhancing grain yield is the main objective of any rice breeding program. Identification of yield and yield-related traits and their introgression into adapted varieties is one of the best strategies to increase grain yield under drought. Several grain yield QTLs in various chromosomes of rice have been identified under drought. *QTL12.1* was the first identified major grain yield QTL in rice under drought (Bernier et al. 2007). Another large effect QTL for grain yield, *qDTY1.1,* was identified on chromosome 1 (Ghimire et al. 2012). Besides these, *qDTY2.2, qDTY3.1, qDTY3.2, qDTY4.1, qDTY6.1, qDTY9.1* were other major effect QTLs identified in rice (Venuprasad et al. 2009,2012b; Swamy et al. 2013; Yadav et al. 2013; Sandhu et al. 2014). The identified QTLs should be consistent in multiple genetic backgrounds and various target environments (Vikram et al. 2011; Prince et al. 2015). Marker assisted pyramiding of the large

effect QTLs in adapted varietal background is necessary to achieve higher grain yield under drought (Shamsudin et al. 2016).

1.8. Rationale and objectives of the research

Rice cultivation requires large amount of water. Scarcity of water and irregular rainfall pattern heavily affect rice production. Exploration of available rice germplasm for drought tolerance is necessary to breed drought tolerant varieties. The identification of QTLs and underlying candidate genes in QTL intervals can be helpful to adopt marker-assisted selection in breeding programs. The overall goal of this study is to generate genomic tools and resources to facilitate drought breeding efforts in USA rice. The specific objectives are as follows:

1. Evaluation of genetic diversity, population structure, and marker-trait association for drought tolerance in rice germplasm of the United States
2. Identification of drought responsive QTLs during vegetative growth stage of rice using a saturated GBS-based SNP linkage map
3. Genetic analysis of yield and agronomic traits under reproductive stage drought stress in rice using a high-resolution linkage map

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Chapter 2. Evaluation of Genetic Diversity, Population Structure, and Marker-Trait Association for Drought Tolerance in Rice Germplasm of the United States

2.1. Introduction

Rice is an important cereal crop both in terms of production and consumption. Nearly half of the world's population relies on rice for their food. Global per capita rice consumption is increasing every year (Mohanty 2013). Various biotic and abiotic factors affect rice production. Rice is a hydrophyte and requires a large amount of water for its growth and development. Since the majority of rice producing areas in Asia and Africa are dependent on rain, drought stress, particularly during reproductive stages, reduces rice yield drastically and threatens food security for millions of people. Droughts in California and water restrictions in Texas have negatively affected rice production in the USA. It shifted the rice acreage to other crops leading to a decline in rice production (Howitt et al. 2015). Drought tolerance studies in rice are necessary to explore the available genetic resources, to understand the mechanism of tolerance, and to develop varieties suitable for water stress environments.

Genetic diversity analysis helps to understand the variability present in rice germplasm. There are an estimated 140,000 diverse rice genotypes in the world. The gene bank in IRRI has preserved ~100,000 genotypes (FAO 2003). These genotypes have several desirable traits, which can be exploited for genetic enhancement. Identification of the rice genotypes with enhanced or stable yield under drought stress is an important prerequisite for breeding drought tolerant varieties. Genetic diversity study unravels such variation among the genotypes and explores the desirable agronomic attributes in them. Principal component analysis and cluster analysis based on the phenotypic attributes can be used to assess the genetic variability (Islam et al. 2018). Plant height, leaf rolling score, grain yield, spikelet fertility, and harvest index are some important traits used in drought screening experiments (Kamoshita et al. 2008).

The environmental influence on phenotypic trait expression limits the utility of genetic diversity studies based on phenotypic traits. On the contrary, assessment of genetic variability using molecular markers is useful in the context of crop improvement. Simple sequence repeat (SSR) markers have been extensively used to evaluate genetic diversity in rice (Das et al. 2013; Anandan et al. 2016). The SSR markers are multi-allelic and can detect more genetic variation compared to SNP (Tabanao et al. 2014) and AFLP markers (Xu et al. 2004). Narrow genetic diversity in USA rice genotypes was observed in a previous study using SSR markers (Lu et al. 2005). However, the study was based on cultivars that have been released in the USA during the 20^{th} century. A comprehensive study of the genetic diversity in all available US germplasm, including the recently developed genotypes, is necessary.

Population structure analysis is a model-based approach that classifies individuals to sub-populations. It helps to identify the admixture or migrants in a population (Pritchard et al. 2000). Identifying the population structure before association analysis reduces type I and type II errors, which may arise due to unequal allele frequency between sub-groups (Pritchard et al. 2000).

Marker trait association is useful to identify the marker loci linked to the traits of interest. It is based on the principle of linkage disequilibrium i.e. non-random association between the alleles at different loci (Anandan et al. 2016). Compared with the bi-parental mapping approach, the association mapping accounts for the recombination events that have been accumulated from the past several generations. Therefore, the results from such type of analysis reflect a larger amount of variation in the population.

There has been no comprehensive study on drought tolerance ability of the USA rice genotypes till date. The objectives of this study were: (i) to screen the USA rice genotype collection for drought tolerance at the reproductive stage under greenhouse conditions, (ii) to

study the genetic variation and population structure in US rice collection, and (iii) to identify markers associated with agronomic traits, yield and yield-attributing traits under drought stress.

2.2. Materials and methods

2.2.1. Plant materials and drought tolerance screening

The present study material consisted of 205 rice genotypes collected from National Genetic Resource Program (NRGS), Louisiana Rice Research Station (LRRS), and International Rice Research Institute (IRRI). Of these, 29 rice genotypes collected from IRRI were developed in different countries around the world. The remaining 176 rice genotypes were developed in various rice growing states of the USA. It included 60 genotypes from Louisiana, 47 from Texas, 34 from Arkansas, 26 from California, 7 from Missouri, and 2 from Mississippi. The details of the genotypes used in the experiment and their states of origin were listed in Appendix Table A1.

The above rice genotypes were screened for drought tolerance at the reproductive stage under greenhouse conditions. The experiment was conducted in a complete randomized design (CRD) with two replications. Plants were grown in 2-gallon plastic pots filled with silty clay soil. Three plants were allowed to grow in each pot. Plants were allowed to grow under ambient conditions with no moisture stress until the emergence of the panicles. Once a plant showed panicle initiation, it was removed from the watered bench and placed in a concrete bench without water. After exposing the plants to drought stress for one week without irrigation, the plants were moved to a bench with water. Various morphological and yield-attributing traits were measured to assess the level of drought tolerance in rice. Days to flowering (DTF) was measured as the number of days from planting to the emergence of the panicle. Number of tillers (NT) in each plant was counted. Leaf rolling score (LRS) was given following the protocol from standard evaluation system of rice (IRRI 2002) in the scale of 1-9. Shoot fresh weight (FW) was measured

as the fresh weight of the above-ground plant. Shoot dry weight (DW) was measured after drying the plant samples in oven at 65^0C for one week. Plant dry matter content (DMC) was measured as the ratio of shoot dry weight to shoot fresh weight and expressed in percentage. Spikelet fertility (SF) was calculated as the ratio of the number of fertile spikelets to the total number of spikelets in a plant and expressed in percentage. Grain yield (GY) was measured in each of the three plants and averaged. Harvest index (HI) was calculated as the ratio of grain yield to shoot dry weight and expressed in percentage.

2.2.2. Genotyping

Eighty SSR markers were used to genotype 184 rice genotypes. DNA was isolated from young leaf tissues of each genotype using CTAB method (Chen and Ronald 1999). Quantification of DNA was done using spectrophotometer (Nanodrop ND-1000, Thermofisher Scientific, MA, USA). A final DNA concentration of 50ng/µl was used for PCR amplification. The PCR reaction mixture contained 3µl of 50ng/µl DNA, 12.8 µl of water, 2.5µl of 10x PCR mixture, 2.5µl each of 25mM $MgCl_2$, and 2mM dNTPs, 1.25µl 50 ng/µl of both forward and reverse primers, and 1U of Taq polymerase (Promega Corporation, Madison, USA). The PCR reaction profile consisted of 35 cycles of the following steps: denaturation at 94^0C for 45s; annealing at 55^0C for 45s (varied depending on the SSR marker); and extension at 72^0C for 1 minute with a final extension at 72^0C for 5 min. The annealing temperature used for PCR reactions was obtained from the Gramene database (http://archive.gramene.org/markers/). The PCR products were run in 4.5% SFR agarose gel and were viewed under UV using gel documentation system.

2.2.3. Statistical analysis

The mean values of the two replications were used for analysis. Mean, standard deviation (SD), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and heritability (h^2) for each of the traits were determined. GCV, PCV, and heritability for each of the traits were calculated in Microsoft excel using the following formulae mentioned in Singh and Chaudhary (1979):

$$GCV = \left(\frac{\sigma_g^2}{\overline{X}} \right)^{\frac{1}{2}} \times 100$$

$$PCV = \left(\frac{\sigma_p^2}{\overline{X}} \right)^{\frac{1}{2}} \times 100$$

$$h^2(\%) = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

where, σ_p^2 = phenotypic variance, σ_g^2 = genotypic variance and $\overline{X}$ = grand mean of the genotypes for the trait of interest.

Correlations among the traits were calculated using Proc Corr procedure in SAS (SAS Inc. 2011). Principal component analysis (PCA) was used to study the relationship among the genotypes and to identify the important variables contributing to the phenotype. Cluster analysis was used to group the genotypes based on the phenotypic traits. PCA and cluster analysis was done using JMP (SAS Inc. 2013).

2.2.4. Genetic diversity and population structure analysis

The presence and absence of the alleles were scored as 1 and 0, respectively. The expected band size of the PCR products was determined from the Gramene website (http://www.gramene.org/marker/). Gene diversity, average number of alleles per locus (AL),

major allele frequency (MAF), and polymorphism information content (PIC) were calculated using PowerMarker software V 3.5 (Liu and Muse 2005). Average number of alleles is the mean of the alleles present in all of the genotypes for a specific marker. Major allele frequency is the relative frequency of the most common allele for a particular marker. PIC was calculated as follows:

$$PIC_i = 1 - \sum_{i=1}^{n} P_i^2$$

Where, 'i' is the i^{th} allele of the j^{th} marker, n is the number of alleles at the j^{th} marker and P is the allele frequency

A model-based program 'Structure 2.2' (Pritchard et al. 2000) was used to assess the population structure. The parameters were set to 50,000 burns-in period followed by 50,000 Markov Chain Monte Carlo (MCMC) simulations. It allowed the admixtures and correlated allele frequencies. The genotypes were classified into sub-populations based on its maximum membership probability. A genotype was considered to be in a sub-population if >70% of its composition came from that group, otherwise it was classified as an admixture. The optimum number of sub-population (K) was determined by running K values from 2 to 10. Each K value was run ten times. True value of K was determined using *adhoc* statistics ΔK proposed by Evanno et al. (2005) in 'Structure harvester' (Earl and Vonholdt 2012).

The unweighted pair group method with arithmetic mean (UPGMA) clustering was done using DARwin software (Perrier and Jacquemoud-Collet 2006). Dissimilarity matrix used for constructing the tree was computed using a shared allele index. The Molecular Analysis of Variance (MANOVA) among the sub-populations identified by structure was computed using GenAlex V 6.5 with 1000 permutations (Peakall and Smouse 2012).

2.2.5. Marker-trait associations

Association of markers with traits was determined using generalized linear model (GLM) and mixed linear model (MLM) in TASSEL 5 (Bradbury et al. 2007). A significant marker trait association was declared when the p-value was less than 0.05.

2.3. Results

Significant variation for all nine traits was observed among genotypes (Table 2.1, Appendix Figure A1). The mean days to flowering for all rice genotypes grown under the greenhouse condition was ~75 days. The average leaf rolling score after exposure to drought was quite high (6.2). The percentage of phenotypic variance explained by our data was greater than 60% for all the variables. Both GCV and PCV were high for yield related traits i.e. spikelet fertility, grain yield, and harvest index. The GCV and PCV for days to flowering were low (12.5% and 12.9%, respectively). High heritability was observed for all the traits. There was a significant positive correlation between days to flowering and yield-related traits (Table 2.2). The number of tillers was negatively correlated with grain yield (-0.13) and harvest index (-0.17). Significant and moderate correlations between leaf rolling score and yield related traits were observed. Plant fresh weight did not correlate significantly with yield. However, plant dry matter content was significantly and negatively correlated with grain yield (-0.28) and other yield related traits.

2.3.1. Principal component analysis

Principal component analysis was done to identify the grouping pattern among the genotypes (Figure 2.1a). The first two principal components accounted for 36% and 31% of the genetic variance (Figure 2.1b). The scree plot indicated that the two principal components would be sufficient to explain most of the variability in the dataset (Figure 2.1c). The eigen values (Appendix Table A2) for three principal components were greater than one. These three

Table 2.1. Estimation of phenotypic and genetic parameters for various agronomic traits, yield, and yield related traits in rice genotypes under drought stress

Traits	Mean	Range	R-square[a]	GCV[b]	PCV[c]	Heritability[d] (%)
Days to flowering	74.9	62-88	0.94	12.5	12.9	93.6
Number of tillers	4.0	2.5-7	0.69	24.7	33.1	55.6
Leaf rolling score	6.2	3-9	0.73	28.2	35.2	64.2
Fresh weight (g/plant)	86.4	29.2-230.4	0.88	48.8	52.5	86.6
Dry weight (g/plant)	38.8	25-93.5	0.91	36.8	38.7	90.2
Plant dry matter content (%)	47.9	13.9-90.0	0.76	28.3	33.8	69.9
Spikelet fertility (%)	30.4	0.1-90.0	0.88	99.1	106.0	87.5
Grain yield (g/plant)	5.7	0.1-46.3	0.87	104.5	112.7	86.0
Harvest index (%)	15.4	0.1-62.4	0.88	103.9	111.4	86.9

[a]Amount of variation explained by the genotypes for the specific trait; [b]Genotypic coefficient of variation; [c]Phenotypic coefficient of variation; [d]Broad sense heritability; [e]Leaf rolling score was measured in the scale of 1-9, 1 being highly tolerant and 9 is highly susceptible

Table 2.2. Pearson correlation coefficients among various agronomic traits, yield, and yield-related traits in rice genotypes under drought stress

	DTF	NT	LRS	FW	DW	PDMC	SF	GY	HI
DTF	1.00	-0.06	-0.19**	0.55**	0.53**	-0.39**	0.19**	0.23**	0.14**
NT		1.00	-0.07	0.29**	0.25**	-0.25**	-0.11	-0.13**	-0.17**
LRS			1.00	-0.17**	0.07	0.52**	-0.37**	-0.45**	-0.46**
FW				1.00	0.84**	-0.66**	-0.07	0.03	-0.11
DW					1.00	-0.30**	-0.21**	-0.13	-0.26**
PDMC						1.00	-0.21**	-0.28**	-0.22
SF							1.00	0.68**	0.70**
GY								1.00	0.93**
HI									1.00

**Significant at 0.01 level of probability; DTF, Days to flowering; NT, Number of tillers; LRS, Leaf rolling score; FW, Fresh plant weight (g/plant); DW, Dry plant weight (g/plant); PDMC, Plant dry matter content (%); SF, Spikelet fertility (%); GY, Grain yield (g/plant); HI, Harvest index (%)

principal components could be used to represent all the variables in the data set. PCA classified the nine variables into three groups (Appendix Table A3). Principal Component 1 (PC1) represented yield related traits *viz.* leaf rolling score, spikelet fertility, grain yield, and harvest index. PC2 indicated shoot related traits such as days to flowering, fresh weight, dry weight, and plant dry matter content and PC3 represented the number of tillers. The most representative variable in group 1 and group 2 were harvest index and fresh weight, which represented 73% and

68% of the variability within the group, respectively. PCA did not show any distinct clustering of the USA genotypes contingent to any state. However, most of the genotypes from California were clustered in quadrant 3.

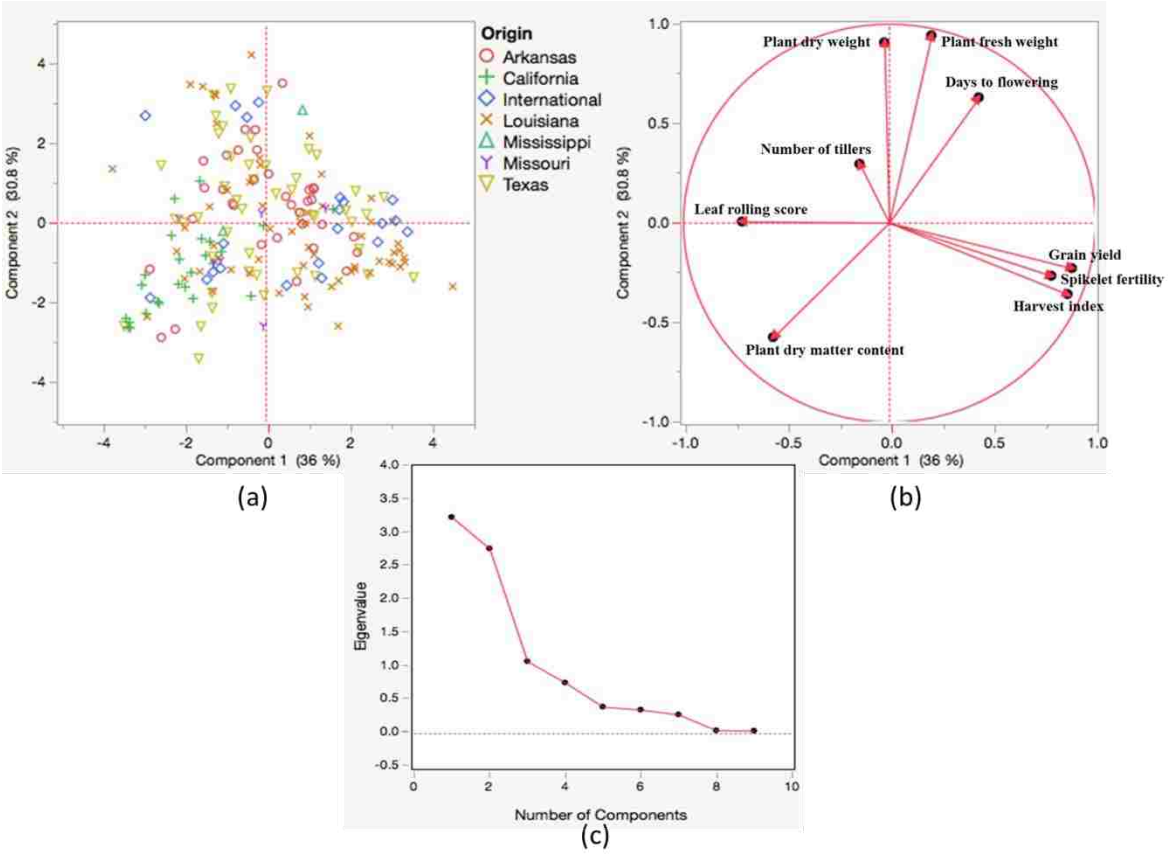

Figure 2.1. Principal component analysis (PCA) plot of various agronomic traits, yield, and yield-related traits in the USA rice genotypes. (a) Scatter plot of the various rice genotypes represented in two major principal component axes. No sufficient clustering was observed except the California genotypes in the third quadrant. (b) Grouping of the variables in two principal components. PC1 represented yield-related traits and PC2 represented the agronomic traits. (c) Scree plot showing the eigen values for all the nine principal components

2.3.2. Cluster analysis

The nine phenotypic traits in rice under drought stress (Table 2.3) classified the genotypes into six clusters (Table 2.4, Figure 2. 2). The level of tolerance of each cluster was assessed by mean values of grain yield and yield-related traits (spikelet fertility and harvest index). The genotypes clustered in group 6 were considered to be highly tolerant under drought stress. The

mean grain yield of genotypes in group 6 was 10.3 g/plant; the spikelet fertility and harvest index were 54.4% and 28.4%, respectively. Group 6 included drought tolerant check genotypes (Dular, SLO16, and Kalia), salt tolerant genotype (Geumgangbyeo), and Bengal. It also included many genotypes from Louisiana, Texas, Arkansas, and California. Group 3 included the rice genotypes that were moderately tolerant to drought. It included drought tolerant checks (N-22, Chengri, Djogolon, and Pin Kaeo) and some USA rice genotypes (Newbonnet, Cypress, and Caffey). The mean spikelet fertility, grain yield, and harvest index for the genotypes in this group were 46%, 6.3g/plant, and 22%, respectively. Tolerant rice genotypes, Early prolific, Rexona, Hybrid mix, and Jefferson, were grouped in Cluster 4. The spikelet fertility, grain yield, and harvest index of the genotypes in this group were 25%, 4g/plant and 11%, respectively. Cluster 1, 2, and 5 contained the genotypes that were susceptible to drought. The mean grain yield for these groups was 2.5, 3, and 1.5 g/plant, respectively. Cluster 5 contained the genotypes, which were most susceptible to drought. It included popular genotypes Alan, Terso, Tauri Mai, Nipponbare, Cocodrie, and Texmont.

Table 2.3. Mean value of each group identified by cluster analysis for agronomic traits, yield, and yield related traits in the USA rice genotypes under drought stress

Group[a]	Count[b]	DTF	NT	LRS	FW	DW	PDMC	SF	GY	HI
1 (S)	36	83.0	3.8	6.8	121.4	52.8	44.7	15.3	2.5	5.9
2 (MS)	37	70.3	4.0	6.9	77.3	33.7	47.5	15.1	3.0	8.4
3 (T)	22	70.1	4.0	6.8	57.2	32.0	57.7	46.0	6.3	21.6
4 (MT)	17	72.4	6.0	6.0	97.0	42.5	47.1	25.4	4.0	11.1
5 (HS)	17	66.5	3.3	8.0	39.4	28.7	73.7	13.1	1.5	5.4
6 (HT)	68	77.2	3.7	4.9	91.6	37.9	42.2	54.4	10.3	28.4

[a]Six different groups identified by cluster analysis: susceptible (S), moderately susceptible (MS), tolerant (T), moderately tolerant (MT), highly susceptible (HS), and highly tolerant (HT); [b]Number of rice genotypes in each group; DTF, Days to flowering; NT, Number of tillers; LRS, Leaf rolling score; FW, Fresh plant weight (g/plant); DW, Dry plant weight (g/plant); PDMC, Plant dry matter content (%); SF, Spikelet fertility (%); GY, Grain Yield (g/plant); HI, Harvest index (%)

Table 2.4. Classification of the USA rice genotypes for drought tolerance based on various agronomic traits, yield, and yield related traits under drought stress

Group (Level of tolerance)	List of rice genotypes
Group 1 (Susceptible)	Starbonnet-1, Rexark-1, Starbonnet-2, Bluebonnet, Toro, Nova, Glutinous selection, FL378, Melrose, Arkansas fortune, Prelude, Rexark Rogue-9262, RD, Nova-66, Stormproof, Carolina Gold, Rexark-2, Lady wright, Sierra, Zenith-2, Epagri, C-4, Tokalon, Texas Patna, TP-49, Moroberekan, Lacrosse, Salvo, Delitus-120, Delitus, Rexark Rogue-9214, Nira-43, Nira, Cheriviruppu, Pokkali
Group 2 (Moderately Susceptible)	Bond, CL162, Tebonnet, S-201, Calrose, Gulfrose, Early Colusa, Vista, M-202, Cheniere, M-102, Jackson, Azucena, LA-0702086, R-52, Sabine, M-301, Calrose-2, Conway, M-201, Catahoula, Bluebelle, Vegold, Bluebelle-2, Pacos, Caloro, MS-1995-15, Gold Zenith, Brazos, Smooth Zenith, Newrex, Kamrose, Colusa, Family-24, Nato, Calady, Skybonnet
Group 3 (Tolerant)	Newbonnet, Cypress, Jazzman-2, Jodon, R-50, Pin Kaeo, N-22, Trenasse, Presidio, Kokubelle, Lafitte, Mermentau, Dixieblle, Palmyra, Rico-1, Early Wataribur, Maybelle, Della-2, Chengri, Kalia-2, Djogolon, Caffey
Group 4 (Moderately Tolerant)	Early Prolific, MS-1996-9, CL261, Hybrid Mix, Lebonnet, Lotus, Damodar, Rexona, S-301, CL111, M-204, CL131, R27, Neches, Lavaoa, Bellemont, Jefferson
Group 5 (Highly Susceptible)	Alan, Terso, Tauri Mai, M-103, Carlpearl, Maxwell, Nipponbare, M-401, Belle Patna, Earlirose, M302, Cocodrie, Millie, Texmont, Gody, Rossmont, Adair
Group 6 (Highly Tolerant)	Zenith, Mars, Arkose selection, Saturn Rouge, Della, Hill Long Grain, Nortai, Cody, Jasmine-85, Evangeline, Dawn, Asahi, Rey, Acadia, CR5272, Saturn, SLO16, Northrose, Bengal, Dellamti, Katy, Taggert, FL478, Lacarus, CL152, MO R-500, Arkose, Gold Nato, Earl, LAH10, LA0802140, CL181, Wells, Templeton, TCCP-266, CL161, Glutinous Zenith, Hill medium, Magnolia, R54, Century Rogue, Toro-2, Short Century, Century Patna, SP14, Orion, CSR-11, Jupiter, Mercury, Dellrose, Geumgangbyeo, CL142, Madison, R-609, Roy J, Neptune, Lacassine, Pirogue, Dellmont, Jazzman, Leah, IRRI147, Ecrevisse, PSVRC, Dular, Jes, Kalia, LA110

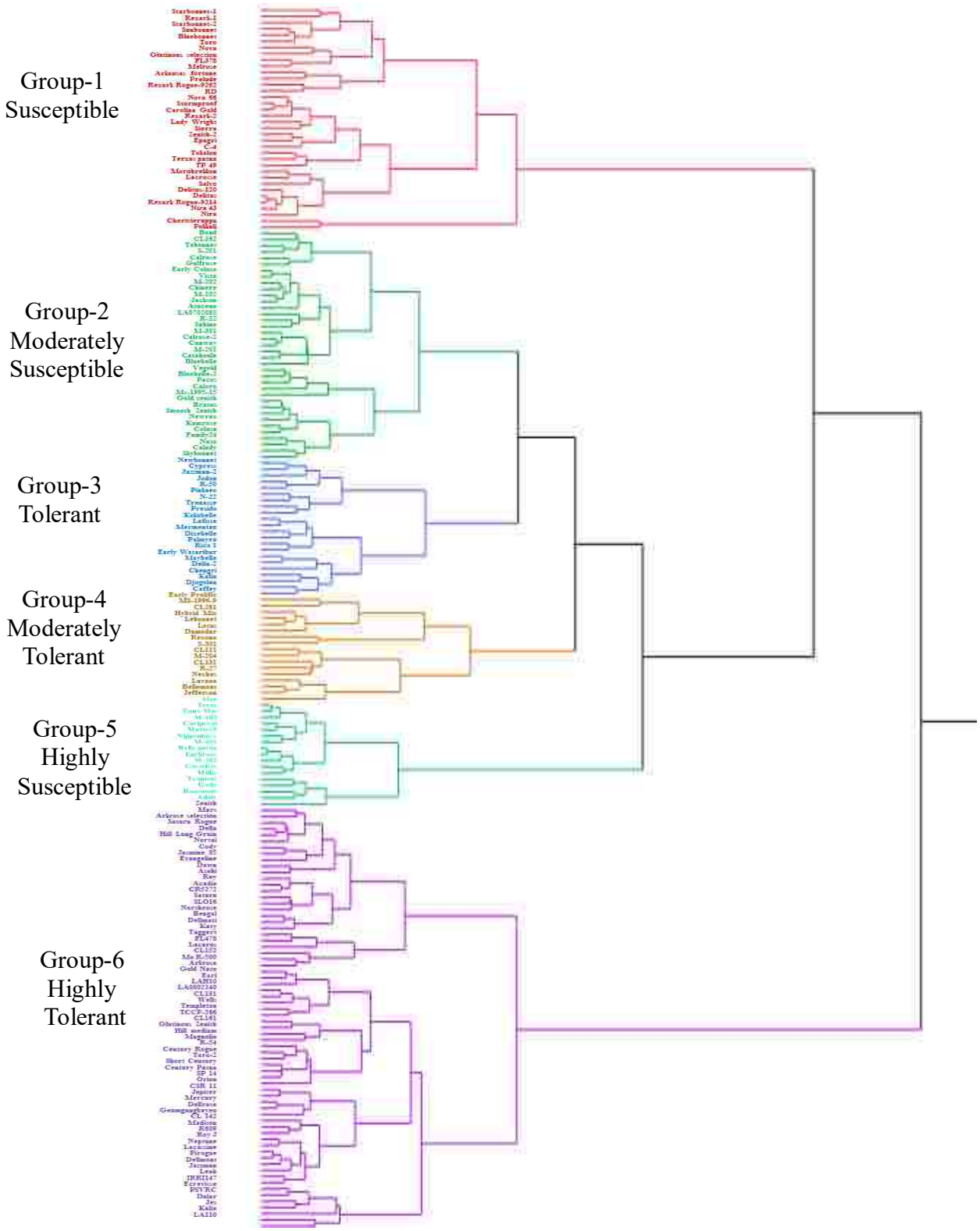

Figure 2.2. Clustering of the rice genotypes based on nine phenotypic traits under drought stress

2.3.3 Genetic diversity

Among the 80 SSR markers used for genotyping, five markers (RM7187, RM192, RM126, RM116, and RM512) were monomorphic (Table 2.5). The maximum number of alleles was 6 in RM8085. A total of 272 alleles were observed with an average of 3.4 alleles per SSR marker. The major allele frequency for the polymorphic markers ranged between 0.39-0.97, with an average of 0.74. The genetic diversity of the markers varied from 0.05 (RM598) to 0.66 (RM8219). Polymorphism information content (PIC) among the polymorphic markers ranged between 0.05 (RM598) to 0.58 (RM488, RM8219, and RM 3428).

Table 2.5. Details of SSR markers used in genotyping of the rice genotypes, major allele frequency, number of alleles, genetic diversity, and PIC values

Marker	Chr.	Position[a] (Mb)	Repeat motif[b]	Product size[c] (bp)	Major allele frequency	No. of alleles	Genetic diversity	PIC[d]
RM259	1	7.4	(CT)17	162	0.81	3	0.33	0.29
RM493	1	12.2	(CTT)9	211	0.73	3	0.43	0.38
RM466	1	17.2	(AG)17	230	0.59	3	0.56	0.49
RM129	1	19	(CGG)8	205	0.83	3	0.30	0.27
RM9	1	23.3	(GA)15GT(GA)2	136	0.78	4	0.36	0.33
RM488	1	24.8	(GA)17	177	0.53	5	0.63	0.58
RM246	1	27.3	(CT)20	116	0.82	6	0.33	0.31
RM302	1	32.9	(GT)30(AT)8	156	0.81	4	0.33	0.30
RM212	1	33	(CT)24	136	0.87	4	0.24	0.23
RM8085	1	34.8	(AG)26	126	0.53	6	0.58	0.49
RM315	1	36.7	(AT)4(GT)10	133	0.76	3	0.39	0.35
RM431	1	38.8	(AG)16	251	0.80	4	0.34	0.31
RM104	1	40.1	(GA)9	222	0.66	4	0.48	0.41
RM84	1	NA	(TCT)10	113	0.87	4	0.23	0.22
RM110	2	1.3	(GA)15	156	0.80	4	0.33	0.30
RM174	2	7	(AGG)7(GA)10	208	0.58	3	0.56	0.48
RM550	2	12.4	(CCT)8	231	0.69	3	0.47	0.42
RM262	2	20.7	(CT)16	154	0.86	3	0.25	0.23
RM13600	2	24.2	(AG)11	122	0.61	5	0.57	0.51

Table 2.5. continued

Marker	Chr.	Position[a] (Mb)	Repeat motif[b]	Product size[c] (bp)	Major allele frequency	No. of alleles	Genetic diversity	PIC[d]
RM263	2	25.8	(CT)34	199	0.72	4	0.45	0.40
RM240	2	31.4	(CT)21	132	0.73	4	0.44	0.40
RM211	2	NA	(TC)3A(TC)18	161	0.85	4	0.26	0.25
RM327	2	NA	(CAT)11(CTT)5	213	0.78	4	0.36	0.34
RM60	3	0.1	(AATT)5AATCT(AATT)	165	0.75	4	0.40	0.36
RM7332	3	0.4	(ACAT)11	205	0.69	2	0.43	0.34
RM523	3	1.3	(TC)14	148	0.59	4	0.58	0.53
RM22	3	1.5	(GA)22	194	0.65	3	0.47	0.38
RM569	3	1.9	(CT)16	175	0.54	4	0.61	0.54
RM517	3	6.1	(CT)15	266	0.67	3	0.45	0.36
RM14980	3	13.9	(AG)17	382	0.84	3	0.27	0.24
RM16	3	23.1	(TCG)5(GA)16	181	0.71	4	0.45	0.41
RM168	3	28.0	T15(GT)14	116	0.8	5	0.34	0.32
RM570	3	35.5	(AG)15	208	0.45	4	0.64	0.57
RM335	4	0.7	(CTT)25	104	0.57	4	0.61	0.56
RM3471	4	6.3	(CT)21	147	0.59	3	0.57	0.51
RM6314	4	18.4	(CTT)11	169	0.82	3	0.30	0.26
RM6250	4	24.8	(CTC)8	187	0.95	2	0.10	0.09
RM7187	4	27.4	(ATAG)7	157	1.00	1	0.00	0.00
RM437	5	3.8	(AG)13	275	0.86	3	0.24	0.23
RM289	5	7.8	G11(GA)16	108	0.86	3	0.25	0.23
RM598	5	16.7	(GCA)9	159	0.97	3	0.05	0.05
RM6054	5	22.7	(CCG)12	128	0.78	4	0.34	0.30
RM274	5	26.8	(GA)15-7-(CGG)5	160	0.84	3	0.28	0.26
RM587	6	2.3	(CTT)18	217	0.77	3	0.38	0.34
RM3	6	19.4	(GA)2GG(GA)25	145	0.87	6	0.23	0.23
RM5371	6	25.8	(TC)13	143	0.76	3	0.38	0.34
RM461	6	30.1	(AAAC)6	195	0.52	3	0.56	0.46
RM314	6	NA	(GT)8(CG)3(GT)5	118	0.95	3	0.10	0.10
RM192	7	0.2	(TGG)5	267	1.00	1	0.00	0.00
RM3449	7	13.4	(CT)19	179	0.72	3	0.44	0.4
RM5793	7	17.4	(AGC)8	127	0.85	4	0.27	0.26
RM351	7	23.9	(CCG)9(CGAAG)4	134	0.71	3	0.45	0.40
RM172	7	29.5	(AGG)6	159	0.51	3	0.56	0.46

Table 2.5. continued

Marker	Chr.	Position[a] (Mb)	Repeat motif[b]	Product size[c] (bp)	Major allele frequency	No. of alleles	Genetic diversity	PIC[d]
RM152	8	0.6	(GGC)10	151	0.72	4	0.44	0.4
RM1376	8	3.1	(AG)31	199	0.59	4	0.58	0.54
RM515	8	20.2	(GA)11	211	0.52	3	0.61	0.53
RM256	8	24.2	(CT)21	127	0.93	3	0.13	0.12
RM126	8	NA	(GA)7	171	1.00	1	0.00	0.00
RM8219	9	1.5	(GA)11	169	0.39	3	0.66	0.58
RM6475	9	12.8	(GCC)9	209	0.67	4	0.51	0.47
RM566	9	14.7	(AG)15	239	0.6	5	0.58	0.53
RM107	9	20	(GA)7	189	0.84	3	0.28	0.26
RM6707	9	22.2	(TAT)8	113	0.79	3	0.33	0.28
RM6862	9	NA	(TGC)9	113	0.78	2	0.34	0.28
RM216	10	5.3	(CT)18	146	0.66	5	0.51	0.46
RM8207	10	9.8	(TTC)23	191	0.52	4	0.63	0.58
RM596	10	15.2	(GAC)10	188	0.67	2	0.44	0.35
RM258	10	18	(GA)21(GGA)3	148	0.85	3	0.27	0.24
RM3451	10	21.5	(CT)19	208	0.77	4	0.37	0.33
RM271	10	NA	(GA)15	101	0.82	4	0.31	0.29
RM26045	11	1.8	(TC)12	297	0.49	3	0.59	0.51
RM116	11	5.7	(CT)9	258	1.00	1	0.00	0.00
RM3428	11	13.4	(CT)18	156	0.42	3	0.65	0.58
RM209	11	17.8	(CT)18	134	0.6	4	0.58	0.53
RM7277	11	24.2	(ATCT)10	148	0.83	2	0.29	0.25
RM7187	11	NA	(AT)29(GT)7	146	0.94	4	0.11	0.11
RM20	12	0.9	(ATT)14	140	0.86	3	0.25	0.23
RM512	12	5.1	(TTTA)5	214	1.00	1	0.00	0.00
RM7195	12	9.9	(ATAG)7	138	0.73	4	0.44	0.40
RM5609	12	23.9	(AAG)9	158	0.84	3	0.28	0.26
Mean					0.74	3.4	0.37	0.33

[a]Physical position of the marker in the chromosome in megabase (Mb); [b]Repeat sequence of the SSR marker; [c]Expected size of the PCR product in base pair (bp); [d]Polymorphism information content

2.3.4. Population structure analysis

The population structure of the rice genotypes was analyzed with software 'Structure' using Bayesian clustering method. The membership fractions of 2-10 were used to classify the genotypes (Appendix Figure A2). The log likelihood LnP (D) and Evanno's deltaK identified eight distinct clusters of the population (Figure 2.3, Figure 2.4). The subgroup 1 (SG1) contained 12 genotypes all of which were *japonica* type. The other genotypes grouped together in this sub-group were admixtures. It contained the genotypes from Arkansas and Texas. Subgroup 2 (SG2) contained 19 genotypes and some admixtures. This subgroup was mostly dominated by the rice genotypes from Texas. All the genotypes in SG2 were *japonica* subspecies. SG3 contained the check genotypes and salt tolerant lines obtained from IRRI. They were mostly of *indica* subspecies. A few Louisiana genotypes and two weedy rice (MS-1995-15 and MS-1996-9) genotypes obtained from Mississippi fell in this subgroup. Fourteen genotypes were under SG4. All of them, except Moroberekan and R-27, were from Louisiana. SG5 contained four genotypes (Delitus-1206, Evangeline, Nira, and Leah) from Louisiana. SG6 contained 26 genotypes from Texas and Louisiana. All sixteen genotypes in SG7 were from Louisiana except Pin Kaeo and Kalia. Among the 20 genotypes in SG8, three (Arkose, Asahi, and Kamrose) were from Arkansas and the others were developed in California.

In the Unweighted pair group method with arithmetic mean (UPGMA) clustering, Louisiana genotypes were separated from other USA rice genotypes (Figure 2.5). The check genotypes obtained from IRRI were highly diverse and did not cluster together with the USA genotypes. The rice genotypes from Arkansas, California, and Texas were grouped together. However, California genotypes were separated from other USA genotypes within the subgroup.

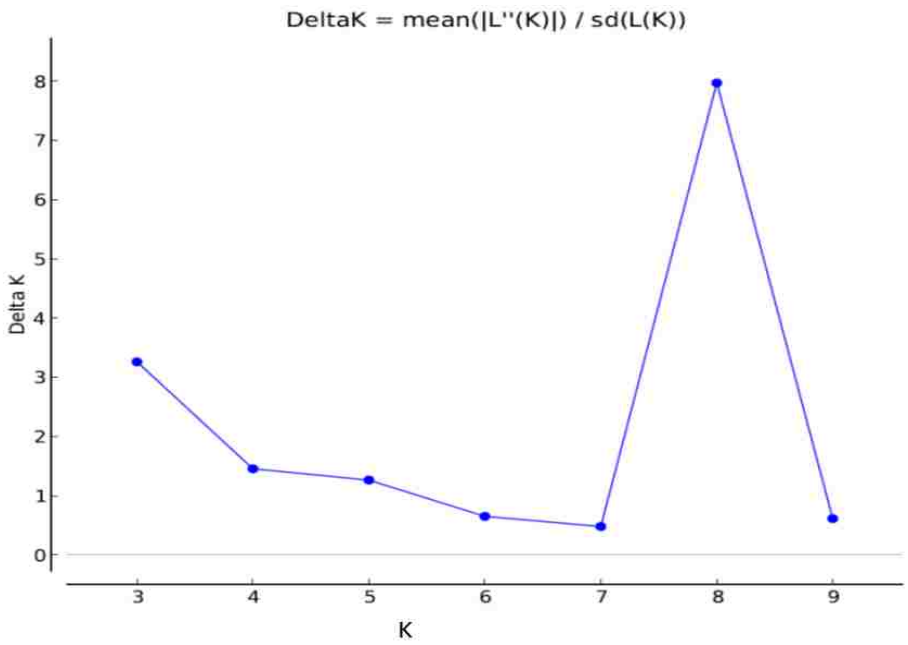

Figure 2.3. Estimation of population structure using LnP(D) derived ΔK for determining the optimum number of subpopulations. The maximum value of delta K was found to be at K=8, which indicated the entire population can be divided into eight subpopulations

2.3.5. Analysis of molecular variance

Eight subpopulation groups obtained by 'Structure' were analyzed for the significant genetic differentiation between and among the groups. AMOVA revealed that 42% of the total variation was among the groups and 58% of the total variation was within the group (Table 2.6). The variation within the group and the variation among the groups were significantly different.

2.3.6. Marker trait association

GLM and MLM revealed 53 and 25 marker-trait association for nine yield and agronomic traits, respectively (Table 2.7). Five marker trait associations were observed for days to flowering using GLM method and two associations were observed using MLM method. RM517 and RM3471 were associated with days to flowering in both methods. RM168, associated with number of tillers, contributed only 3% of the phenotypic variance (PV). GLM and MLM together

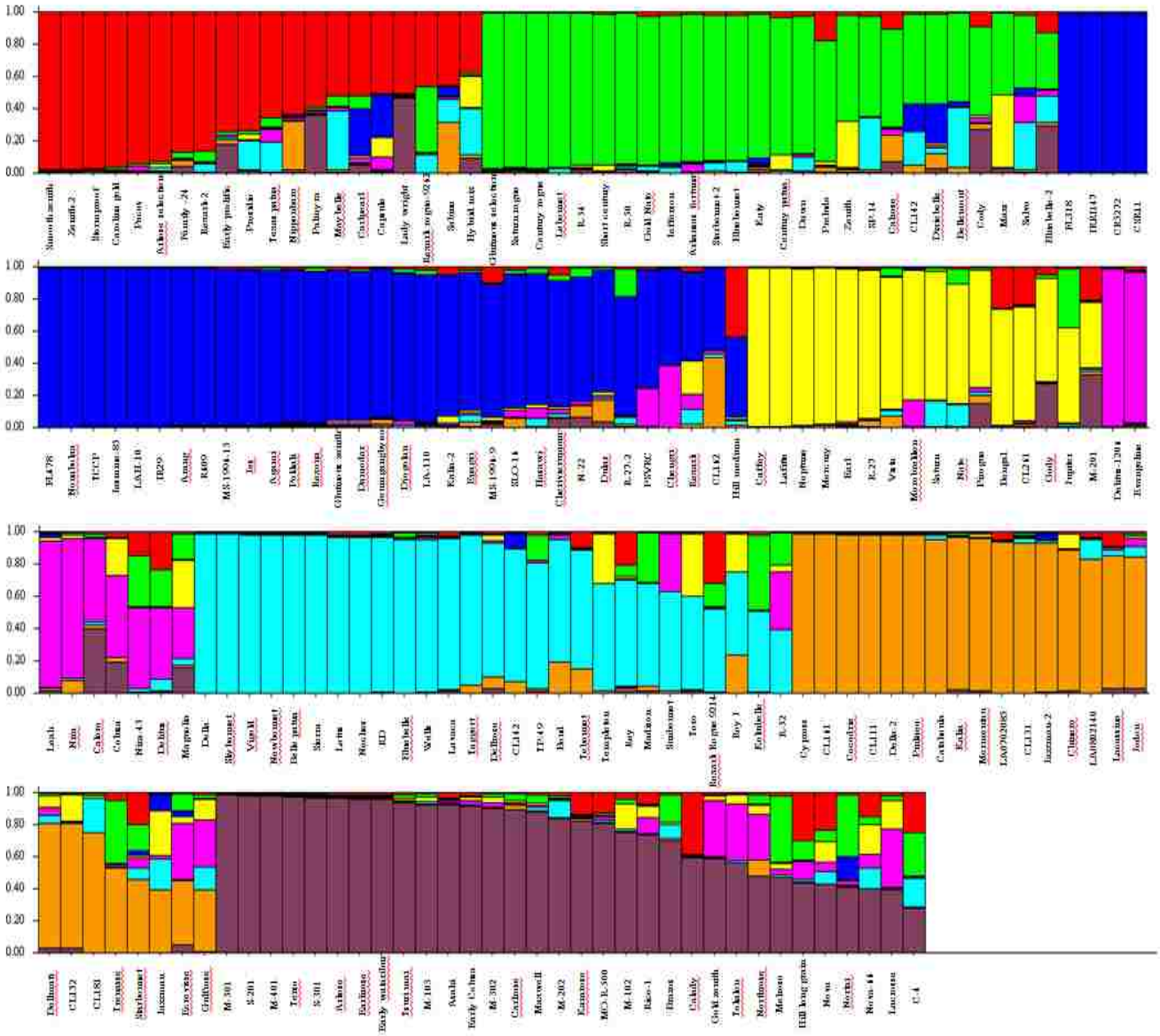

Figure 2.4. The estimated population structure of rice genotypes (K=8). The y-axis corresponded to the subgroup membership and the x-axis represented the genotype. The genotypes with the probability of ≥70% were assigned to a specific subgroup, while the others were classified as admixtures.

detected four markers (RM129, RM351, RM256, and RM216) linked to leaf rolling score. RM351 in chromosome 7 showed a strong association (6% PV) with leaf rolling score. Besides these, GLM detected RM 129, RM152, and RM216, which contributed 5% of the genetic variation for leaf rolling score. There were five markers that were significantly associated with shoot fresh weight, in both GLM and MLM methods. RM302 in chromosome 1 located at 33 Mb

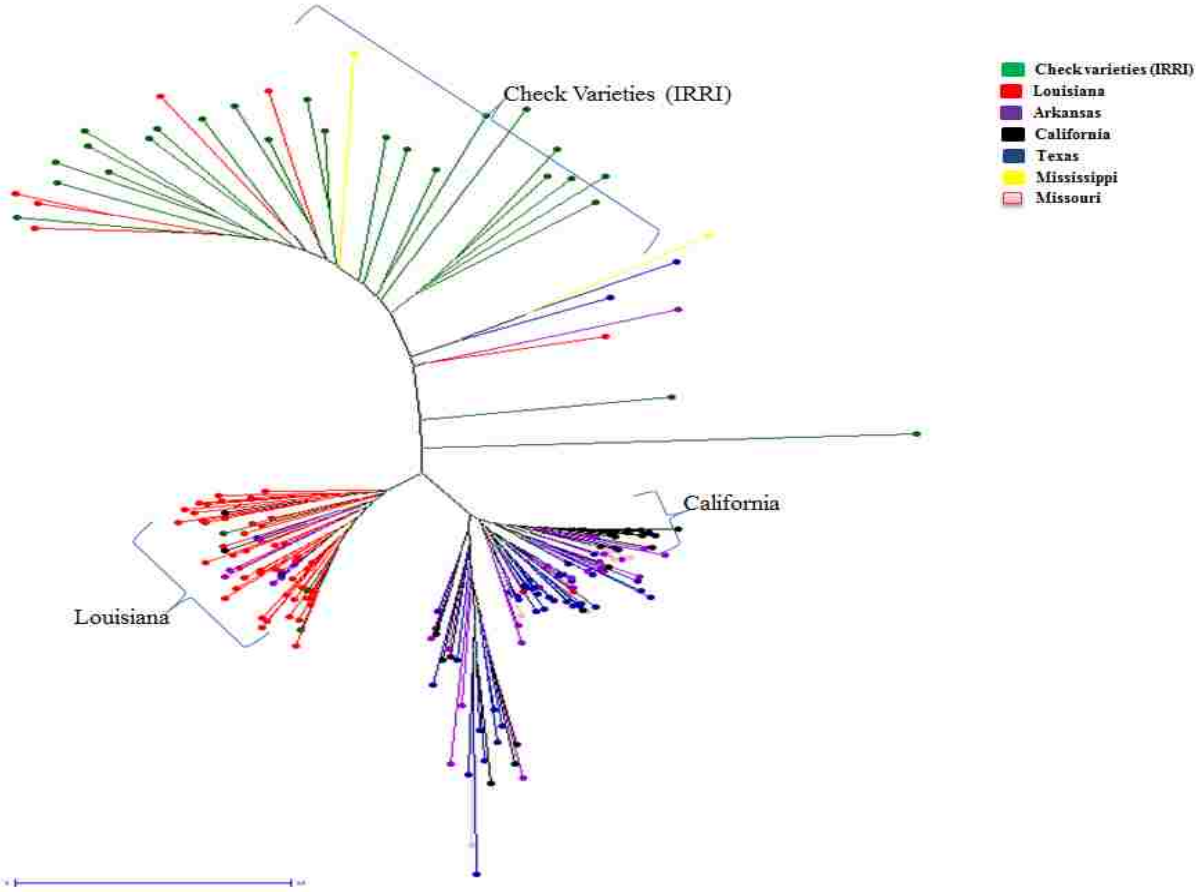

Figure 2.5. Unweighted pair group method with arithmetic mean (UPGMA) tree of rice genotypes using SSR markers. Different color codes represented rice genotypes developed in various states in the USA. Check genotypes were developed in different countries of the world and were obtained from IRRI.

Table 2.6. Analysis of molecular variance (AMOVA) among the eight sub-populations identified by 'Structure' software

Source of variation	DF[a]	SS[b]	MSS[c]	Estimated variance	% variance	P-value[c]
Among Population	7	790.77	112.96	6.34	42	<0.0001
Within Population	124	1095.75	8.84	8.84	58	<0.0001
Total	131	1886.52		15.18	100	

[a]Degree of freedom; [b,]Sum of squares; [c]Mean sum of squares; [d]Level of significance

position showed 5% phenotypic variation for fresh weight. For dry weight, GLM and MLM detected 14 and 5 markers, respectively. RM302, RM3471, RM461, and RM8207 explained 9%, 6%, 8%, and 7% of the phenotypic variability, respectively for dry weight in GLM analysis. RM315 showed association with plant dry matter content in both methods. Both GLM and MLM

Table 2.7. Significant marker trait association in rice genotypes under drought stress using GLM (Q) and MLM (Q+K) model

Traits	Marker	Chr.	Pos.	GLM[a] (Q) Model			MLM[b] (Q+K) Model		
			(Mb)	F-value	P-value[c]	R-square[d]	F-value	P-value[c]	R-square[d]
Days to flowering	RM246	1	27.3	6.46	0.01	0.03			
	RM22	3	1.5	8.12	<0.01	0.03	7.61	0.01	0.04
	RM517	3	6.2	5.00	0.03	0.02			
	RM335	4	0.7	4.38	0.04	0.02			
	RM3471	4	6.3	10.25	<0.01	0.05	4.87	0.03	0.03
No. of Tillers	RM168	3	28.1	4.80	0.03	0.03	4.16	0.04	0.03
Leaf rolling score	RM129	1	19.0	9.23	<0.01	0.05	4.26	0.04	0.02
	RM168	3	28.1	7.69	0.01	0.04			
	RM570	3	35.6	8.35	<0.01	0.04			
	RM351	7	23.9	10.53	<0.01	0.06	5.25	0.02	0.04
	RM152	8	0.7	10.60	<0.01	0.05			
	RM256	8	24.3	4.54	<0.01	0.02	5.51	0.02	0.03
	RM566	9	14.7	4.98	0.03	0.02			
	RM216	10	5.4	11.27	<0.01	0.05	4.38	0.04	0.02
	RM7195	12	9.9	4.68	0.03	0.03			
Fresh weight	RM302	1	33	10.27	<0.01	0.05	10.1	<0.01	0.05
	RM431	1	38.9	6.08	0.01	0.03	6.81	0.01	0.04
	RM3471	4	6.3	5.50	0.02	0.03	4.32	0.04	0.02
	RM289	5	7.8	4.72	0.03	0.02	5.62	0.02	0.03
	RM5371	6	25.8	6.06	0.01	0.03	4.22	0.04	0.02
	RM1376	8	3.2	5.25	0.02	0.03			
	RM566	9	14.7	4.58	0.03	0.02			
Dry weight	RM129	1	19.0	5.98	0.02	0.03			
	RM302	1	33.0	20.95	<0.01	0.09	5.74	0.02	0.04
	RM212	1	33.1	4.66	0.03	0.02			
	RM262	2	20.8	4.48	0.04	0.02			
	RM14980	3	13.9	7.17	0.01	0.03			
	RM570	3	35.6	7.58	0.01	0.03			
	RM3471	4	6.3	11.72	<0.01	0.06	8.43	<0.01	0.05
	RM289	5	7.8	6.39	0.01	0.03	6.82	0.01	0.04
	RM587	6	2.3	4.98	0.03	0.02	3.98	0.05	0.02
	RM3	6	19.5	4.56	0.03	0.02			
	RM5371	6	25.8	7.54	0.01	0.03			
	RM461	6	30.1	9.77	<0.01	0.08	8.45	<0.01	0.09
	RM351	7	23.9	8.37	<0.01	0.04			
	RM8207	10	9.8	7.48	0.01	0.07			
Plant dry matter content	RM315	1	36.7	6.60	0.01	0.03	6.27	0.01	0.04
	RM6054	5	22.8	4.33	0.04	0.02			

Table 2.7 continued

Traits	Marker	Chr.	Pos.	GLM[a] (Q) Model			MLM[b] (Q+K) Model		
			(Mb)	F-value	P-value[c]	R-square[d]	F-value	P-value[c]	R-square[d]
Spikelet fertility	RM431	1	38.9	6.09	0.01	0.03	4.44	0.04	0.03
	RM168	3	28.1	5.23	0.02	0.03			
	RM570	3	35.6	11.76	<0.01	0.06	7.25	0.01	0.04
	RM6054	5	22.8	8.95	<0.01	0.04	7.17	0.01	0.04
	RM351	7	23.9	6.68	0.01	0.04	5.02	0.03	0.03
	RM216	10	5.4	5.29	0.02	0.03			
Grain Yield	RM523	3	1.3	5.38	0.02	0.03			
	RM517	3	6.2	5.50	0.02	0.03			
	RM570	3	35.6	5.80	0.02	0.03	4.12	0.04	0.03
	RM351	7	23.9	6.56	0.01	0.04			
	RM256	8	24.3	5.47	0.02	0.03			
Harvest Index	RM523	3	1.3	5.41	0.02	0.03			
	RM570	3	35.6	5.27	0.02	0.03	4.04	0.04	0.03
	RM598	5	16.8	4.12	0.04	0.02			
	RM351	7	23.9	10.51	<0.01	0.06	5.23	0.02	0.07

[a]Generalized linear model; [b]Mixed linear model (MLM accounts for the population structure and kinship matrix); [c]Level of significance; [d]Variance contributed by the marker

detected four markers associated with spikelet fertility. RM 570 located in 35.6 Mb region of chromosome 3 contributed 6% of the phenotypic variance for spikelet fertility in GLM model. GLM detected five markers associated with grain yield. RM570 was detected in both the methods. Four marker trait associations were detected for harvest index in rice using GLM method. MLM detected two markers (RM570 and RM351) associated with harvest index. RM351 showed a strong association with harvest index with a phenotypic variance of 6% and 7% by GLM and MLM methods, respectively.

2.4. Discussion

Exploring the available genetic resources is the first step in any successful breeding program. Drought tolerance studies in the USA rice germplasm have been given the least attention until now. However, with the current trend of climate change, drought is becoming a major threat to crop production, especially in rice. Drought increases the days to flowering, reduces the plant height, and reduces spikelet fertility, resulting in reduced grain yield (Kamoshita et al. 2008;

Ndjiondjop et al. 2010; Sandhu and Kumar 2017; Bhattarai and Subudhi 2018a). Leaf rolling is an important first indicator for measuring drought responsiveness in rice (Prince et al. 2015; Bhattarai and Subudhi 2018b). A high average leaf rolling score of USA rice germplasm indicated their susceptibility under drought. Correlation studies indicated that leaf rolling score negatively affected grain yield and spikelet fertility in rice. Therefore, early selection for yield could be done by scoring the leaf rolling under drought stress. A reduction in spikelet fertility observed under drought stress was responsible for the drastic reduction in grain yield and harvest index. The yield reduction of >50% under drought stress was reported in earlier studies (Vikram et al. 2011; Swamy et. al. 2017). The genotypic coefficient of variation and the phenotypic coefficient of variation indicated a wide variation in yield, spikelet fertility, and harvest index. It indicated that the USA rice germplasm collection include both drought tolerant and susceptible genotypes. High heritability for grain yield and yield attributing traits under drought stress implied that these traits could be used as primary selection criteria in any drought screening experiment (Kumar et al. 2014). Heritability values of >70% for days to flowering and grain yield had been reported earlier (Vikram et al. 2011; Dixit et al. 2014; Swamy et al. 2017). The heritability for grain yield under drought stress was high compared to that of non-stress (Swamy et al. 2017).

High degree of variation for drought tolerance in USA rice germplasm was evident from the principal component analysis. The variation in drought tolerance among rice genotypes was not dependent on the state of origin, except for the rice genotypes from California. Most of the California genotypes were separated from the rice genotypes from other states and were more susceptible to drought. PCA further clarified that the three variables i.e. harvest index, fresh weight, and number of tillers, were sufficient to capture most of the variation in the data. These

three important traits could be used to screen the rice genotypes for drought tolerance. Furthermore, cluster analysis of the USA rice genotypes grouped them according to their level of drought tolerance. Tolerant genotypes had high spikelet fertility and grain yield under drought compared to that of the susceptible ones. The rice genotypes from various states grouped under the drought tolerant category included Zenith, Mars, Arkrose, Asahi, Katy (from Arkansas), Dawn, Madison, Hill Long Grain (from Texas), MO R-500 (from Missouri), Rey, Della, Acadia, Saturn, Bengal, Dellmati, Taggert, Magnolia, Wells, Templeton (from Louisiana). The inclusion of CL142, CL111, and Mermentau under the drought tolerant group in our study was in agreement with an earlier study (Singh et al. 2017). Some deep rooting genotypes like Moroberekan and Azucena were clustered in the susceptible group. This may be due to the fact that these deep rooting genotypes were not able to penetrate into the soil in the pot experiment. Some salt tolerant rice genotypes like Damodar, Cypress, Caffey, Jupiter, and Jazzman (De Leon et al. 2015) showed drought tolerance under the greenhouse conditions. The rice genotypes showing both salt and drought tolerance may be due to similar physiological responses and co-expression of the genes under both stress conditions (Nounjan et al. 2018).

The rice genotypes from six major rice growing states of the USA were evaluated for genetic diversity using molecular markers. SSR markers were useful to identify small allelic variation among the individuals (Tabanao et al. 2014). The average PIC in our study was 0.33 which was less compared to the average PIC of 0.54 in the global rice collection (Zhang et al. 2011). The PIC value with a range of 0.21 - 0.50 was observed in USA rice in previous studies (Xu et al. 2004; Lu et al. 2005). A lower PIC value indicated the presence of low genetic diversity in USA rice germplasm (Xu et al. 2004; Islam et al. 2018). The genetic diversity of *japonica* subspecies was small compared to its *indica* counterpart. The average PIC of the global *japonica* rice

collection was 0.42 and the European collection of tropical *japonica* rice was 0.37 (Courtois et al. 2012). The dominance of *japonica* subspecies in USA rice germplasm collection may be the reason for low genetic diversity compared to the global collection.

A model-based approach of population structure identified eight subgroups. Previous studies in rice had identified two to eight subpopulations (Nachimuthu et al. 2015; Anandan et al. 2016; Pradhan et al. 2016; Islam et al. 2018). The threshold to identify a genotype into a specific subgroup varied from 60-80%. Our stringent threshold of 70% similarity, to be in a specific group, identified 49 genotypes as admixtures. Population structure analysis separated the check genotypes of *indica* group from the *japonica* USA genotypes. The rice genotypes from California, Louisiana, and Arkansas were different from each other, whereas the rice genotypes from Texas appeared to be a mixture of the rice genotypes from Louisiana and Arkansas. Few rice genotypes from Arkansas matched closely with the California rice genotypes. It was concluded that the USA rice genotypes could be classified into three major groups depending on the state of origin i.e. Louisiana, Arkansas, and California. These differences in rice genotypes from the three major rice-growing states may be due to the different rice growing ecosystems prevailing in those regions. A similar study in US weedy rice showed variation in the rice genotypes according to the region of origin (Shivarin et al. 2010).

The result of the model-based structure analysis agreed to the results from the UPGMA tree. It indicated that the USA rice genotypes were completely different from the Asian rice genotypes (Lu et al. 2005) as they were separated distinctly from each other. The two weedy rice genotypes from Mississippi (MS-1995-15 and MS1996-9) were closer to the *indica* type check genotypes.

Marker-trait association studies have been implemented in many germplasm collections to identify the molecular markers linked with a trait of interest (Pradhan et al. 2016; Swamy et al.

2017). The marker RM22, associated with days to flowering under drought stress, co-localized with previously identified QTLs *qDTF3.01* (Bhattarai and Subudhi 2018a), *DTY3.2* (Vikram et al. 2016) and heading date locus *hd9* (Lin et al. 2012). RM246 was linked to days to flowering and photosynthetic rate in rice (Ramchander et al. 2016). This explained the probable correlation between the photosynthetic rate and the days to flowering. A previously identified marker RM517 controlling the DTF QTL *(qDTF3.3)* (Vikram et al. 2016) was identified in our study. RM152 was correlated with the leaf rolling score, which was associated with leaf water content and stomatal conductance in rice under drought stress (Ramchander et al. 2016). RM216 co-localized with the previously identified QTL *qDTY10.1* (Vikram et al. 2011). RM302 was linked with plant fresh weight and plant dry weight with a phenotypic variance of 9%. This marker co-localized with the previously identified QTL for leaf water content (Ramchander et al. 2016) suggesting dependence of plant biomass on leaf water content. Both plant biomass and leaf water content were highly correlated with drought tolerance in rice. The two novel markers (RM8207 and RM461), identified in this study with a contribution of 7-8% toward phenotypic variance, were responsible for plant dry weight under stress. The markers, RM212 and RM262, which co-localized with the previously identified QTLs for plant height (Prince et al 2015) and grain yield (Swamy et al. 2017), were associated with plant dry weight under stress. The markers associated with grain yield and yield components could be used directly to select plants for yield. RM431, closely linked to QTL *qDTY1.1* (Venuprasad et al. 2012), was related to spikelet fertility. A new marker, RM351, on chromosome 7 was associated with spikelet fertility, grain yield, and harvest index with a phenotypic variance of 7%. RM523 and RM570 were the two important markers controlling grain yield and harvest index in rice. These novel markers could be used in drought breeding program for direct selection of yield.

2.5. Conclusions

Drought in rice is a major constraint in rice growing areas of the USA and other parts of the world. It is predominantly grown in lowland, well-watered condition. However, the disturbance in the global climate is limiting the availability of water for agricultural purpose. Since drought tolerance studies in the USA rice germplasm are limited, the drought tolerant USA genotypes identified in this study will be useful for breeding drought tolerant rice varieties. A low genetic diversity observed in USA rice germplasm calls for introduction of the new diverse germplasm to enhance genetic diversity. The molecular markers that were associated with the gain yield and agronomic traits under drought stress will be useful for marker-assisted breeding to develop varieties with enhanced yield and stability under drought prone areas.

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Chapter 3. Identification of Drought Responsive QTLs During Vegetative Growth Stage of Rice Using a Saturated GBS-Based SNP Linkage Map

3.1. Introduction

Rice is predominantly grown in well-watered conditions. But it is also grown in upland and rainfed lowlands, which constitutes 45% of the global rice production area. The majority of the rice growing areas in Asia and Africa are dependent on rain (Hijmas and Serraj 2009). The yield reduction in rice grown in these drought-affected areas is estimated to be 44-71% (Pandey and Bhandari 2009). Therefore, development of drought tolerant rice varieties is necessary to improve food security on a global scale.

Drought may occur at any growth stage of the crop. Early season drought occurs at the vegetative stage of growth and affects leaf growth and stem elongation. Intermittent drought occurring in between the rainfall intervals affects the development of the root system. Terminal drought occurs at the end of the growing period particularly during the flowering stage, affecting grain filling and spikelet fertility (Kamoshita et al. 2008). There are three mechanisms of plant's tolerance to drought stress i.e. drought escape, drought avoidance, and drought tolerance (Basu et al. 2016). Efforts are made to incorporate these mechanisms for developing drought tolerant rice varieties using both conventional and molecular breeding approaches.

Identification of QTLs for drought tolerance is a preferred breeding strategy to develop varieties tolerant to drought. However, the cost involved in phenotyping and genotyping of large number of individuals in breeding programs has been a significant bottleneck. The stage of plant growth, target environment, and the intensity of drought are important factors to be considered while identifying the QTLs for drought tolerance in rice (Kamoshita et al. 2002). Several QTLs

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have been identified for drought tolerance traits at both seedling and reproductive stages (Srividhya et al. 2011; Vikram et al. 2011; Venuprasad et al. 2012; Palanog et al. 2014; Sandhu et al. 2014; Saikumar et al. 2014; Prince et al. 2015; Swamy et al. 2017). Consistent and large effect QTLs for yield under drought stress are necessary for use in breeding program. Prince et al. (2015) identified large effect yield QTLs on chromosomes 1, 4, and 6 which can be introgressed to stabilize rice productivity under drought stressed environments. Identification of drought responsive QTLs in seedling stage is more focused on root and shoot traits. Deep root traits in plants are responsible for tolerance to a drought environment and increased yield (Uga et al. 2013). The study on drought tolerance should therefore include identification and incorporation of both root and shoot trait QTLs during seedling, vegetative and reproductive growth stages of the plant.

Precise phenotyping of traits associated with drought tolerance is required for precise identification of QTLs. Two types of phenotyping strategies have been suggested by Blum (2002). The first strategy includes phenotyping of the constitutive traits like flowering time, stay green traits, and root depth etc. The second one involves phenotyping of the stress responsive traits under drought stress. It includes accumulation of osmolytes, membrane thermostability, and leaf water content etc. Drought resistance mechanism in rice includes both drought tolerance via osmotic adjustment and drought avoidance aided by a deep root system (Zhang J. et al. 2001).

Roots are important plant organs for plant growth and survival. Proper growth of root is essential in rice during seedling establishment, nutrient uptake, and water absorption. Longer root length, higher root biomass, and increased root shoot ratio are necessary for plants to

increase the water absorption capacity and remain productive under drought stress (Comas et al. 2013). QTLs governing the root traits under drought stress in rice have been identified by various researchers (Champoux et al. 1995; Ali et al. 2000; Zhang J et al. 2001; Zhang WP et al. 2001; Courtois et al. 2003). A deep root system combined with tissue tolerance and shorter growth duration is an important drought avoidance strategy in rice (Champoux et al. 1995; Dixit et al. 2014). Both additive and epistatic QTLs are important in controlling root traits in rice (Zhang WP et al. 2001). Pleiotropic effects have been reported for some highly correlated traits (Ali et al. 2000). Therefore, selection for root traits can be done by selecting other secondary traits.

Identifying the best marker system for QTL analysis is crucial in any molecular breeding program. Various markers systems (RFLP, RAPD, SSR, and AFLP etc.) have been used to map many agronomic traits on the rice genome (Gowda et al. 2003). However, these marker systems are labor-intensive, technically demanding, and are not amenable for generating markers in large number for constructing a saturated linkage map. Availability of whole genome sequence information of rice made it easy to generate unlimited number of markers in rice. Single nucleotide polymorphism (SNP) marker is now preferred by many researchers for genotyping in breeding programs due to increased efficiency and cost effectiveness (Swamy and Kumar 2013). Genotyping by sequencing (GBS) is amenable for generating large number of markers, which can be used to prepare a saturated genetic map to identify QTLs with narrow confidence intervals (De Leon et al. 2016). In this study, we mapped the QTLs related to root and shoot traits in a recombinant inbred line (RIL) population developed from the cross Cocodrie x N-22 under drought stress at the vegetative stage using a high-density GBS-based SNP linkage map and identified several potential candidate genes involved in enhancing drought tolerance.

3.2. Materials and methods

3.2.1. Plant materials and phenotyping

A RIL population was developed from a cross involving two rice varieties, N-22 and Cocodrie. Cocodrie is a drought susceptible US variety released by the Louisiana State University Agricultural Center (Linscombe et al. 2000) and N-22 is a well-known donor for drought tolerance (Vikram et al. 2011). The F_1 plants were selfed to generate F_2 generation which was then advanced by single seed descent method to obtain $F_{7:8}$ RILs. One hundred and eighty-one RILs and two parents were phenotyped during the vegetative growth stage for root and shoot traits inside a plastic tunnel at Louisiana State University during the summer of 2015 (June-July) (Appendix Figure B1). Seventy-five-centimeter-long plastic pots were used to allow maximum root growth of plants. The pots were filled with sand and soil (1:1) and placed vertically in a 30cm deep tray filled with water. Five seeds were planted in each pot and allowed to grow for one week. After one week, three plants per pot were retained to grow until the measurements were taken. The experiment was conducted in a completely randomized design with two replications. There were two sets of experiments: control experiment, where plants were allowed to grow in well-irrigated condition and stress experiment, where drought stress was imposed. After five weeks of plant growth, water was drained out from the tray in the stress experiment and irrigation was withheld for 10 days. Measurements were taken on 45-day old plants. The plants along with soil were taken out of the pot and washed with water to remove soil from the roots. Root length was measured from the base of the culm to the tip of the root. Shoot length was measured from the base of the culm to the tip of the plant. Fresh root mass was measured immediately after washing the roots and draining the residual water from it. Fresh shoot mass was measured by detaching the shoot from the plant. Dry root and shoot mass were

measured after oven drying the samples at 65^{o}C for 5 days. Numbers of tillers were counted. Root-shoot ratio was derived as the ratio of dry root weight to dry shoot weight.

3.2.2. Data analysis

The mean value from each pot was used for analysis. The distributions of all the traits were visualized by making the frequency distribution graphs in Microsoft Excel 2010. SAS 9.3 was used for statistical analysis (SAS Institute 2011). Descriptive statistics were obtained using PROC MEANS procedure in SAS. Analysis of variance was done using PROC GLLIMIX in SAS with line as a fixed effect and replication as a random effect. Pearson correlation coefficients among the traits was computed for both stressed and non-stressed environment using PROC CORR procedure in SAS. Broad-sense heritability was estimated on family mean basis using the SAS code of Holland et al. (2003).

3.2.3. DNA Isolation and sequencing

Leaf samples were collected from 21-day old seedlings of each of the 181 RILs and parents (Cocodrie and N-22). DNA was isolated using modified CTAB method (Murray and Thomson 1980) and purified using Genomic DNA clean and concentrator (Zymo Research Corp. CA, USA) following manufacturer's instruction. DNA quantity was assessed using NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA) and the samples were diluted to 30-100 ng/µL for library construction. *ApeKI* restriction enzyme was used for library preparation following a protocol modified from Elshire et al. (2011). Library preparation and single-end sequencing of the GBS libraries were done at the Genomic Diversity Facility of the Cornell University.

3.2.4. Sequence data analysis and SNP identification

The raw sequence was analyzed using Tassel 3 GBS pipeline (Glaubitz et al. 2014). A Tassel plugin was used to remove the raw sequences without having a barcode. Good barcoded reads were aligned with the reference sequence using Bowtie 2 (Langmead and Salzberg 2012). SNP calling and filtering were done using the Tassel pipeline. The duplicate SNPs were merged and the SNPs with low coverage and high heterozygosity were removed. SNPs having a high rate of genotyping error or not in linkage disequilibrium (LD) with other nearby SNPs on the same chromosome were purged. The SNPs retained after filtering using Tassel pipeline were subjected to manual filtering. The heterozygous SNPs were encoded as missing. SNPs with monomorphic allele between the two parental lines were discarded. All the SNP markers with >10% of the missing value were removed manually before analysis. A total of 4748 SNP markers were retained for linkage map construction.

3.2.5. Linkage map construction and QTL analysis

Linkage map construction and QTL analysis were done using QTL ICIMapping 4.1 (Meng et al. 2015). Since our mapping population is a RIL population, we used '2' to represent the Cocodrie allele, '0' to represent the N-22 allele and '-1' for any missing data. The grouping of the SNP markers was based on their physical map location on the chromosomes. The ordering of the markers within each chromosome was based on the recombination events between the markers. Recombination distance was calculated using Kosambi mapping function (Kosambi 1944).

Eight phenotypic traits were used for QTL mapping. Mean phenotypic data was used for analysis. Interval mapping (IM) and Inclusive Composite Interval mapping (ICIM) were used to detect the additive QTLs. A logarithm of odds (LOD) value greater than 2 was used to declare

the significant QTLs. Since rice is a diploid species and the average chromosome length in our experiment was ~140cM, the LOD value 2 is sufficient to explain almost 95 percent of the probability of being a significant QTL in that region (Ooijen 1999). A LOD value greater than 3 was used to declare the significant epistatic QTLs. The left and the right markers flanking the QTLs were determined. QTLs were named according to the trait name, chromosome number, and their physical map position on the genome. For example, *qSL1.38* represents QTL for shoot length on chromosome 1 at 38 Mb position of the genome. The parental source of QTL effect was determined from the additive effects of the QTLs.

3.2.6. Identification of candidate gene and gene ontology

The position of SNP markers flanking the QTL regions was used against the MSU rice reference genome annotation release 7.0 to identify the genes present within the QTL regions. All the genes within the identified QTLs were listed. These gene lists for each trait were annotated into different gene ontology groups using agriGO gene ontology (GO) analysis toolkit (Tian et al. 2017). The significant gene ontology terms were classified into three major functional groups: biological process, molecular function, and cellular component.

3.3. Results

3.3.1. Phenotypic performance

There was a significant difference in performance between the parents in both stressed and non-stressed environments for all traits (Table 3.1). Mean values for all the traits were higher in N-22 compared with Cocodrie and distributions of these traits were normal (Figure 3.1). Transgressive segregation of RILs was observed on both sides of the distribution. There were significant differences among the RILs for all the traits. Heritability for all the traits was high in both environments.

Root length of Cocodrie remained the same in both stressed and non-stressed conditions whereas it was increased in N-22 under water stress (Appendix Figure B2). The mean root lengths of the RILs lie between the parents in both environments. Both parents experienced a reduction in shoot length, fresh and dry shoot mass under water stress. N-22 showed a greater reduction in shoot length compared with Cocodrie. The mean and range of the RIL population for shoot length were smaller under water stress than in the non-stress environment. N-22 had greater fresh root mass and shoot mass compared to Cocodrie under both environments. Although both parents and the RIL population showed a reduction in fresh root mass under drought stress, the reduction was greater in N-22. The number of tillers increased under water stress for N-22; however, it was decreased for Cocodrie and the RIL population. Dry shoot mass was reduced in both parents under water stress condition. N-22 and RIL mean showed a decrease in dry root mass under water stressed condition compared to well-irrigated condition. Root-shoot ratio increased for both the parents and RIL population under drought stress.

3.3.2. Correlations among the traits

Significant correlation was observed among many of the traits studied (Table 3.2). Root length was significantly and positively correlated to most of the traits in both conditions with the exception of number of tillers and root shoot ratio under drought stress. However, number of tillers and root-shoot ratio were significantly correlated to root length only under non-stress condition. Shoot length, fresh root mass, fresh shoot mass, dry root mass, and dry shoot mass were significantly and positively correlated to each other in both the conditions. Number of tillers was negatively correlated to shoot length but uncorrelated to root-shoot ratio under drought stress. The root-shoot ratio was negatively correlated to dry shoot mass and positively correlated to dry root mass under drought stressed environment.

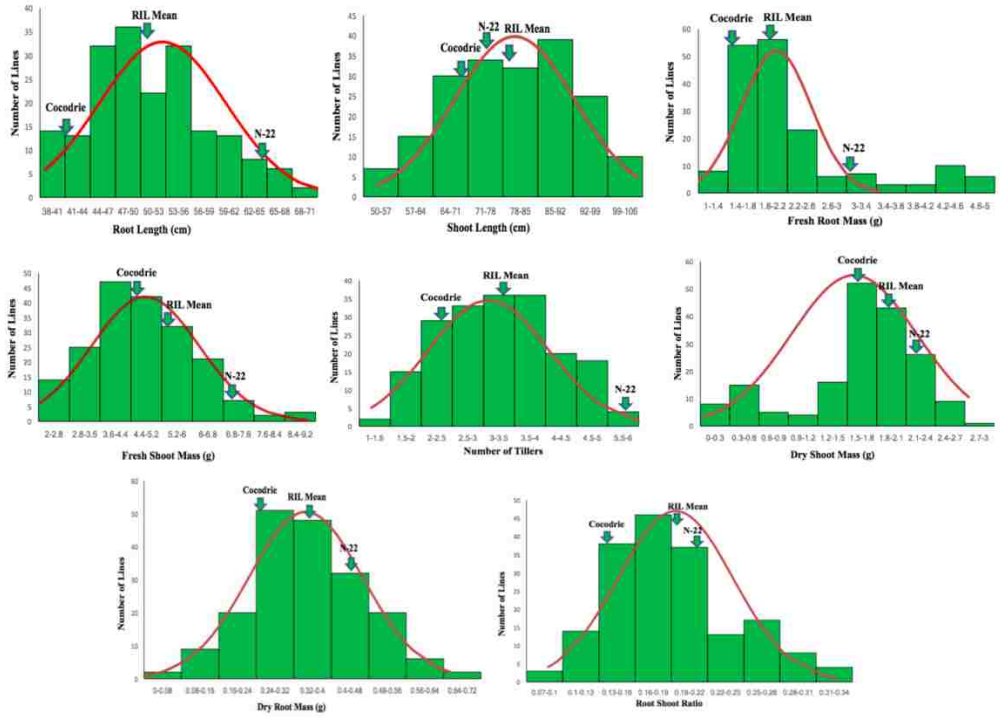

Figure 3.1. Frequency distribution of various root and shoot traits under water stress condition in Cocodrie x N-22 RIL F8 population

3.3.3. GBS and Linkage map construction

The molecular linkage map, constructed using 4748 SNP markers, covered 365 Mb of rice genome with a total genetic length of 1693cM (Table 3.3). The average length of chromosome per SNP was 141 cM with 2.8 markers per cM. There were 2007 recombination points along the whole rice genome with 2.4 SNPs per unique recombination points. A total of 19 gaps greater than 5 cM length were found along 12 rice chromosomes of which the maximum number of 4 gaps was on chromosome 1.

3.3.4. QTL mapping

Inclusive composite interval mapping (ICIM) identified fourteen QTLs for various roots and shoot traits in rice under water stress condition (Table 3.4; Figure 3.2) and nineteen QTLs under irrigated condition (Table 3.5). Twenty-seven QTLs were identified by interval mapping

(Table 3.6) under water stress condition and thirty-one QTLs were identified under irrigated condition (Table 3.7).

3.3.4.1. Root length QTLs

Both IM and ICIM mapping detected one QTL (*qRL12.04*) for root length in chromosome 12 under water stressed environment (Table 3.4; Table 3.6). The QTL explained 5% of the total phenotypic variation and the allele with increased effect was contributed by N-22. Two QTLs for root length, *qRL2.04* and *qRL1.08*, identified under non-stress condition (Table 3.5) explained 7% and 5% of the phenotypic variation, respectively, and the desirable alleles for both the QTLs were contributed by N-22. There were four pairs of epistatic QTLs for root length identified by interval mapping (Appendix Table B1). None of those epistatic QTLs co-localized with the additive QTLs. Among four pairs of epistatic QTLs, three of those have the mean increasing effect from N-22 allele and only Cocodrie contributed the trait enhancing allele for *qRL6.28*. These epistatic QTLs contributed 9% of the total phenotypic variation.

3.3.4.2. Shoot length QTLs

Five shoot length QTLs were identified under water stressed condition. The *qSL1.38* was detected in both IM and ICIM methods and it accounted for 19% and 35% of the phenotypic variation, respectively. Among the shoot length QTLs under drought stress, *qSL1.37*and *qSL4.06* were the major effect QTLs with a high LOD score contributing 36% and 18% of the total phenotypic variation, respectively. The allele with increasing mean effect was contributed byN-22 for *qSL1.37* whereas it was Cocodrie for *qSL4.06*. Four additive QTLs were detected for shoot length under non-stress condition. Nine pairs of epistatic QTLs were identified for shoot length. One of the additive QTL *qSL4.25* co-localized with the epistatic QTL. These epistatic

Table 3.1. Phenotypic characterization of parents and RIL population developed from the cross Cocodrie x N-22 for various root and shoot traits under drought stress and non-stress environments

Trait[a]	Non-Stressed					Stressed					% reduction in trait mean under stress[c]	
	Cocodrie mean	N-22 mean	RIL mean	RIL Range	h^{2b}	Cocodrie mean	N-22	RIL mean	RIL Range	h^2	Cocodrie	N-22
RL (cm)	41.75	53.00*	47.39	17.2-74.0	0.72	41.00	64.50**	50.89	28.7-78.6	0.86	1.80	-21.7
SL (cm)	82.75	103.00*	92.15	33.1-142.2	0.80	70.05	73.65[ns]	79.66	40.6-119.5	0.90	15.35	29.35
FRM (g)	1.64	3.27**	2.38	1.0-4.7	0.76	1.45	3.08**	2.08	1.0-5.9	0.84	11.59	5.81
FSM (g)	6.10	12.74**	8.79	2.9-23.5	0.72	4.05	7.02*	5.20	0.7-15.2	0.81	33.61	44.9
NT	2.84	4.83**	3.97	1.0-8.7	0.65	2.33	5.84**	3.43	1.0-7.0	0.67	17.96	-20.91
DSM (g)	2.22	3.20*	3.03	0.6-8.4	0.77	1.69	2.23*	1.90	0.8-3.7	0.72	23.87	30.31
DRM (g)	0.19	0.50**	0.40	0.03-1.37	0.86	0.24	0.46**	0.37	0.07-0.83	0.80	26.32	8.00
RSR	0.08	0.16**	0.13	0.03-0.47	0.81	0.14	0.21**	0.19	0.05-0.69	0.73	-75.00	-31.26

[a] RL, Root length; SL, Shoot length; FRM, Fresh root mass; FSM, Fresh shoot mass; NT, Number of tillers; DSM, Dry shoot mass; DRM, Dry root mass; RSR, Root shoot ratio; CV, coefficient of variation. [b] h^2: Broad sense heritability on family mean basis.*, **: Significant difference between the means of Cocodrie and N-22 at 5% and 1% level of probability, respectively. [ns] not significant [c] Negative values indicate increase in trait means.

Table 3.2. Pearson correlation matrix among various root and shoot traits in the Cocodrie x N-22 RIL population under drought stress and non-stress environments. Values above diagonal are the correlation among traits under non-stress condition and values below diagonal are the correlations among traits under drought stress condition

Trait[a]	RL (cm)	SL (cm)	FRM(g)	FSM (g)	NT	DSM (g)	DRM (g)	RSR
RL (cm)	**1.00**	0.17*	0.47***	0.53***	0.28***	0.45***	0.46***	0.31***
SL (cm)	0.25***	**1.00**	0.22**	0.30***	-0.12	0.46***	0.37***	0.12
FRM (g)	0.26***	0.21**	**1.00**	0.57***	0.42***	0.51***	0.52***	0.32***
FSM (g)	0.38***	0.32***	0.57***	**1.00**	0.43	0.63***	0.54***	0.26***
NT	0.07	-0.31***	0.26***	0.42***	**1.00**	0.27***	0.38***	0.31***
DSM (g)	0.23**	0.54***	0.40***	0.51***	0.15*	**1.00**	0.58***	0.07
DRM (g)	0.16*	0.34***	0.21**	0.23**	0.15*	0.41***	**1.00**	0.80***
RSR	0.04	0.02	-0.03	-0.07	0.09	-0.23**	0.71***	**1.00**

[a] RL, Root length; SL, Shoot length; FRM, Fresh root mass; FSM, Fresh shoot mass; NT, Number of tillers; DSM, Dry shoot mass; DRM, Dry root mass; RSR, Root shoot ratio. * significant at 0.05 level of probability; ** significant at 0.01 level of probability; *** significant at <0.001 level of probability.

Table 3.3. Summary of the SNP markers distribution and genome coverage in the linkage map of the Cocodrie x N-22 RIL population

Chr	No. of SNP Markers	Chromosome length coverage (bp)[a]	Genetic length (cM)[b]	No. of recombination points	No. of SNP markers/cM	No. of SNP markers/unique position	Minimum interval (cM)	Maximum interval (cM)	Average interval (cM)	No. of gaps >5cM
1	711	43219493	238.8	278	2.98	2.56	0.30	7.54	0.86	4
2	596	35384138	161.9	210	3.68	2.84	0.30	8.86	0.77	2
3	509	35756527	187.1	258	2.72	1.97	0.30	4.73	0.73	0
4	288	35464762	158.9	148	1.81	1.95	0.31	7.20	1.07	3
5	320	29187384	120.9	158	2.65	2.02	0.30	6.34	0.77	1
6	357	29702863	141.1	157	2.53	2.27	0.30	9.74	0.90	1
7	394	29327089	141.6	165	2.78	2.38	0.30	7.42	0.86	1
8	279	28081119	125.1	137	2.23	2.04	0.30	7.32	0.91	1
9	354	22348084	98.8	144	3.58	2.46	0.30	7.94	0.69	1
10	310	22051113	95.8	115	3.23	2.70	0.30	5.09	0.83	1
11	306	27689871	111.9	113	2.73	2.71	0.30	5.80	0.99	2
12	324	27095052	110.9	124	2.92	2.61	0.30	9.44	0.90	2
Total	4748	365307495	1692.8	2007	33.86	28.51	3.61	87.42	10.28	19
Mean	395.7	30442291.3	141.1	167.3	2.82	2.38	0.30	7.28	0.84	1.58

[a] Physical length of chromosome in base pairs (bp).

[b] Length of the chromosome based on recombination events and measured in centimorgan (cM).

QTLs showed 3-9% of the phenotypic variation. The alleles for four of the nine epistatic QTLs were contributed by N-22 and the alleles for other five epistatic QTLs were from Cocodrie.

3.3.4.3. Fresh root mass QTLs

One additive QTL (*qFRM1.36),* detected by both IM and ICIM for fresh root mass under water stress condition, explained 7% of the total phenotypic variation and the desirable allele was contributed by N-22. Two QTLs (*qFRM1.37 and qFRM7.11*) for fresh root mass under non-stress condition accounted for 11% and 5% of the phenotypic variation, respectively. Ten pairs of epistatic QTLs were found for fresh root mass. The alleles with increasing effect for all of these epistatic QTLs were from N-22. None of the additive QTL for fresh root mass co-localized with the epistatic QTLs.

3.3.4.4. Fresh shoot mass QTLs

One additive QTL (*qFSM8.11*) explaining 5% of the total phenotypic variation for fresh shoot mass was identified on chromosome 8 by both IM and ICIM mapping under water stress condition. The increasing mean effect for this QTL was contributed by Cocodrie allele. Four additive QTLs were identified for fresh shoot mass during irrigated condition with each explaining 4-8% of the total phenotypic variation. Seven pairs of epistatic QTLs were detected for fresh root mass. None of these epistatic QTLs co-localized with the additive QTLs. Four and five pairs of interacting QTLs have increasing effects from N-22 and Cocodrie, respectively.

3.3.4.5. Number of tillers QTLs

Two additive QTLs on chromosome 3 were detected for number of tillers under drought stress. Each QTL explained 7% of the phenotypic variation and the allele for increasing mean effect in these QTLs were contributed by N-22. Two pairs of epistatic QTLs were detected for number of tillers in rice under drought. N-22 allele contributed toward increased effect at both of

Table 3.4. Additive QTLs for various root and shoot related traits in the Cocodrie x N-22 RIL population under water stressed condition identified by ICIM

Trait[a]	QTL[b]	Chr	Position (cM)	Left Marker	Right Marker	Interval Size (bp)	LOD[c]	PVE (%)[d]	Additive effect	Number of genes in the QTL	Parental allele with increasing effect
RL	*qRL12.04*	12	30	S12_4402981	S12_4833513	430532	2.2	5.1	-2.35	49	N-22
SL	*qSL1.11*	1	68	S1_11086521	S1_11356196	269675	2.2	1.6	1.90	38	Cocodrie
	qSL1.38	1	188	S1_38023681	S1_38286772	263091	30.4	35.8	-8.87	43	N-22
	qSL3.24	3	109	S3_24014766	S3_24061769	47003	2.7	2.1	2.18	9	Cocodrie
	qSL4.06	4	32	S4_6504436	S4_6630807	126371	17.6	17.9	6.23	19	Cocodrie
	qSL4.29	4	115	S4_29179250	S4_29482850	303600	3.2	2.6	2.40	43	Cocodrie
FRM	*qFRM1.36*	1	180	S1_36461341	S1_36027561	433780	2.6	6.7	-0.15	65	N-22
FSM	*qFSM8.11*	8	53	S8_11845128	S8_15630955	3785827	2.2	5.4	0.40	539	Cocodrie
NT	*qNT3.34*	3	180	S3_34874041	S3_35150742	276701	3.3	6.4	-0.19	52	N-22
	qNT3.28	3	135	S3_28513305	S3_28809504	296199	3.4	6.8	-0.19	44	N-22
DRM	*qDRM1.11*	1	68	S1_11086521	S1_11356196	269675	2.4	5.5	0.03	38	Cocodrie
	qDRM1.37	1	184	S1_37376121	S1_37561874	185753	3.4	7.6	-0.04	35	N-22
	qDRM8.25	8	104	S8_25099054	S8_25341501	242447	2.2	4.4	-0.03	35	N-22
DSM	*qDSM1.38*	1	188	S1_38023681	S1_38286772	263091	4.9	11.3	-0.13	43	N-22

[a] RL, Root length; SL, Shoot length; FRM, Fresh root mass; FSM, Fresh shoot mass; NT, Number of tillers; DRM, Dry root mass; DSM, Dry shoot mass.

[b] *qRL*, *qSL*, *qFRM*, *qFSM*, *qNT*, *qDRM*, and *qDSM* are QTLs for root length, shoot length, fresh root mass, fresh shoot mass, number of tillers, dry root mass, and dry shoot mass, respectively. The number before the decimal indicate chromosome and the number after decimal indicate the physical location of the QTL in mega base pair.

[c] LOD: Logarithm of odds

[d] PVE (%): Percentage of phenotypic variance explained by the QTL

Table 3.5. Additive QTLs for various root and shoot related traits in the Cocodrie x N-22 RIL population under irrigated condition as identified by ICIM

Trait [a]	QTL[b]	Chr.	Position (cM)	Left Marker	Right Marker	Interval size (bp)	LOD[c]	PVE (%)[d]	Additive effect	No. of genes in the QTL	Parental allele with increasing effect
RL	*qRL1.08*	1	49	S1_8361311	S1_8402167	40856	2.9	5.0	-2.18	7	N-22
	qRL2.04	2	22	S2_4310292	S2_4408126	97834	3.4	8.4	-2.82	15	N-22
SL	*qSL1.14*	1	80	S1_14612053	S1_17898781	3286728	2.1	3.0	2.94	477	Cocodrie
	qSL1.38	1	188	S1_38023681	S1_38286772	263091	23.4	42.3	-10.29	43	N-22
	qSL4.32	4	132	S4_32621161	S4_33263832	642671	3.3	4.6	3.38	100	Cocodrie
	qSL5.23	5	93	S5_23719865	S5_23790439	70574	2.3	3.0	-2.70	14	N-22
FRM	*qFRM1.37*	1	182	S1_37089296	S1_37273187	183891	5.1	11.2	-0.22	25	N-22
	qFRM7.11	7	54	S7_11034818	S7_12924619	1889801	2.6	5.4	0.15	258	Cocodrie
FSM	*qFSM1.38*	1	190	S1_38286772	S1_38611845	325073	3.7	7.8	-0.76	50	N-22
	qFSM3.13	3	90	S3_13604998	S3_16656021	3051023	3.2	6.6	0.70	450	Cocodrie
	qFSM5.20	5	73	S5_20111573	S5_20319917	208344	2.6	5.4	-0.63	26	N-22
	qFSM10.11	10	41	S10_11045261	S10_11388953	343692	2.3	4.4	0.59	43	Cocodrie
DRM	*qDRM2.03*	2	19	S2_3854717	S2_4310292	455575	2.0	3.2	-0.04	72	N-22
	qDRM3.30	3	157	S3_30663783	S3_30800456	136673	2.2	3.2	-0.04	26	N-22
	qDRM6.10	6	58	S6_10836601	S6_11170855	334254	3.9	6.0	0.06	48	Cocodrie
	qDRM6.06	6	34	S6_6463372	S6_7403500	940128	6.7	10.8	-0.08	135	N-22
DSM	*qDSM1.37*	1	182	S1_37089296	S1_37273187	183891	4.6	11.8	-0.30	25	N-22
	qDSM7.05	7	110	S7_5450298	S7_5566085	115787	2.0	4.3	0.18	24	Cocodrie
RSR	*qRSR2.03*	2	17	S2_3854717	S2_4310292	455575	3.9	8.2	-0.02	73	N-22

[a] RL: Root length, SL: Shoot length, FRM: Fresh root mass, FSM: Fresh shoot mass, NT: Number of tillers, DRM: Dry root mass, DSM: Dry shoot mass, RSR: Root shoot ratio.

[b] The number before the decimal indicate chromosome and the number after decimal indicate the physical location of the QTL in mega base pair.

[c] LOD: Logarithm of odds.

[d] PVE (%): Percentage of phenotypic variance explained by the QTL.

Table 3.6. Additive QTLs for various root and shoot related traits identified by Interval Mapping (IM) in Cocodrie x N-22 RIL population under water stressed condition

Phenotype	QTL[a]	Chr	Position (cM)	Left Marker	Right Marker	Interval Size (bp)	LOD [b]	PVE (%)[c]	Additive effect	Number of genes in the QTL	Parental allele with increasing effect
Root Length-IM	*qRL12.04*	12	30	S12_4402981	S12_4833513	430532	2.2	5.1	-2.35	49	N-22
Shoot length-IM	*qSL1.37*	1	182	S1_37089296	S1_37273187	183891	17.4	16.0	-8.02	25	N-22
	qSL1.38	1	188	S1_38023681	S1_38286772	263091	22.3	19.3	-8.75	43	N-22
	qSL1.39	1	200	S1_39692180	S1_39526933	165247	9.9	9.7	-6.16	25	N-22
	qSL4.25	4	97	S4_25750452	S4_27194959	1444507	2.6	2.8	3.31	226	Cocodrie
	qSL7.005	7	1	S7_553805	S7_612387	58582	2.7	2.9	3.38	8	Cocodrie
	qSL7.03	7	24	S7_3920648	S7_4483382	562734	3.3	3.8	3.87	79	Cocodrie
	qSL9.03	9	4	S9_3884948	S9_4427876	542928	2.5	2.7	3.23	73	Cocodrie
Fresh Root Mass-IM	*qFRM1.22a*	1	92	S1_22128076	S1_22191567	63491	2.5	4.4	0.22	7	Cocodrie
	qFRM1.22b	1	98	S1_22796323	S1_22958631	162308	2.2	3.8	0.20	23	Cocodrie
	qFRM1.36	1	180	S1_36461341	S1_36027561	433780	2.7	4.7	-0.16	65	N-22
	qFRM5.28	5	119	S5_28710628	S5_28744011	33383	2.0	3.6	-0.14	5	N-22
Fresh Shoot Mass-IM	*qFSM8.11*	8	53	S8_11845128	S8_15630955	3785827	2.2	5.4	0.40	539	Cocodrie
No. of Tillers-IM	*qNT3.34*	3	180	S3_34874041	S3_35150742	276701	2.0	3.7	-0.17	52	N-22
	qNT3.28	3	135	S3_28513305	S3_28809504	296199	3.4	6.3	-0.22	44	N-22
	qNT3.27	3	126	S3_27263281	S3_27364861	101580	3.2	5.8	-0.21	15	N-22
	qNT3.06	3	35	S3_6156903	S3_6332631	175728	2.3	4.1	0.17	26	Cocodrie
	qNT4.05	4	30	S4_5728245	S4_6059987	331742	2.7	5.1	-0.19	52	N-22
	qNT5.23	5	89	S5_23200302	S5_23218617	18315	2.7	4.9	0.19	1	Cocodrie
Dry Root Mass-IM	*qDRM1.21*	1	89	S1_21626281	S1_21068987	557294	2.4	5.2	0.04	72	Cocodrie
	qDRM1.38	1	188	S1_38023681	S1_38286772	263091	2.7	6.1	-0.03	43	N-22
	qDRM8.25	8	104	S8_25099054	S8_25341501	242447	2.3	4.9	-0.03	36	N-22

Table 3.6. continued

Phenotype	QTL[a]	Chr	Position (cM)	Left Marker	Right Marker	Interval Size (bp)	LOD [b]	PVE (%)[c]	Additive effect	Number of genes in the QTL	Parental allele with increasing effect
Dry Shoot Mass-IM	*qDSM1.28*	1	134	S1_28234412	S1_28190405	44007	2.6	3.7	-0.10	4	N-22
	qDSM1.36	1	180	S1_36461341	S1_36027561	433780	4.5	6.4	-0.13	65	N-22
	qDSM1.38	1	188	S1_38023681	S1_38286772	263091	4.9	7.1	-0.13	43	N-22
	qDSM8.08	8	47	S8_8971655	S8_9136647	164992	2.2	3.2	0.09	20	Cocodrie
Root Shoot ratio-IM	*qRSR10.11*	10	41	S10_11045261	S10_11388953	343692	3.0	8.5	-0.02	42	N-22

[a] qRL, qSL, qFRM, qFSM, qNT, qDRM, qDSM, and qRSR are QTLs for root length, shoot length, fresh root mass, fresh shoot mass, number of tillers, dry root mass, dry shoot mass, and root shoot ratio, respectively. The number before the decimal indicate chromosome and the number after decimal indicate the physical location of the QTL in mega base pair; [b] LOD is logarithm of odds; [c] PVE (%) is percentage of phenotypic variance explained by the QTL

Table 3.7. Additive QTLs for various root and shoot related traits identified by Interval Mapping (IM) in the Cocodrie x N-22 RIL population under irrigated condition

Phenotype	QTL[a]	Chr.	Position (cM)	Left Marker	Right Marker	Interval size (bp)	LOD[b]	PVE (%)[c]	Additive effect	No. of genes in the QTL	Parental allele with increasing effect
Root Length - IM	*qRL2.04*	2	22	S2_4310292	S2_4408126	97834	3.4	7.1	-2.82	15	N-22
Shoot Length - IM	*qSL1.21*	1	91	S1_21230560	S1_21995746	765186	2.9	2.4	5.32	110	Cocodrie
	qSL1.33	1	168	S1_33239950	S1_33415088	175138	5.1	4.2	-5.35	22	N-22
	qSL1.35	1	178	S1_35776217	S1_35761539	-14678	13.4	9.9	-8.30	1	N-22
	qSL1.37	1	182	S1_37089296	S1_37273187	183891	14.9	11.1	-8.81	25	N-22
	qSL1.38	1	189	S1_38023681	S1_38286772	263091	21.6	14.9	-10.18	41	N-22
	qSL1.39	1	200	S1_39692180	S1_39526933	165247	6.7	5.3	-6.06	25	N-22
Fresh Root Mass- IM	*qFRM1.37*	1	182	S1_37089296	S1_37273187	183891	3.9	6.8	-0.20	25	N-22
	qFRM1.38	1	190	S1_38286772	S1_38611845	325073	3.8	6.5	-0.19	51	N-22
Fresh Shoot Mass-IM	*qFSM1.36*	1	181	S1_36252166	S1_37068548	816382	2.8	4.8	-0.70	119	N-22
	qFSM1.38	1	190	S1_38286772	S1_38611845	325073	3.2	5.5	-0.75	51	N-22
	qFSM3.16	3	89	S3_16260951	S3_16599892	338941	2.4	4.8	0.71	40	Cocodrie
	qFSM3.12	3	72	S3_12741486	S3_12847784	106298	2.2	3.7	0.61	20	Cocodrie
	qFSM6.06	6	34	S6_6463372	S6_7403500	940128	2.1	3.6	-0.60	134	N-22
Number of Tillers - IM	*qNT3.31*	3	158	S3_31005190	S3_31490364	485174	3.3	4.7	-0.26	71	N-22
	qNT6.27	6	116	S6_27149332	S6_27427622	278290	3.5	5.1	-0.27	46	N-22
	qNT6.26	6	111	S6_26499660	S6_26675063	175403	3.3	5.0	-0.27	23	N-22
	qNT6.25	6	106	S6_25146090	S6_25717883	571793	3.1	4.7	-0.26	89	N-22
	qNT6.24	6	95	S6_24332630	S6_24728241	395611	2.7	4.2	-0.25	48	N-22

Table 3.7. continued

Phenotype	QTL[a]	Chr.	Position (cM)	Left Marker	Right Marker	Interval size (bp)	LOD[b]	PVE (%)[c]	Additive effect	No. of genes in the QTL	Parental allele with increasing effect
Dry Root Mass – IM	*qDRM2.03*	2	20	S2_3854717	S2_4310292	455575	2.1	3.8	-0.05	73	N-22
	qDRM3.30	3	157	S3_30663783	S3_30800456	136673	2.2	3.7	-0.05	26	N-22
	qDRM3.16	3	91	S3_16459035	S3_16450935	8100	3.5	5.9	0.06	1	Cocodrie
	qDRM3.13	3	78	S3_13860899	S3_13948553	87654	3.8	6.5	0.07	10	Cocodrie
	qDRM6.06	6	34	S6_6463372	S6_7403500	940128	3.3	5.6	-0.06	135	N-22
Dry Shoot Mass - IM	*qDSM1.37*	1	182	S1_37089296	S1_37273187	183891	4.8	8.1	-0.32	25	N-22
	qDSM1.38	1	190	S1_38286772	S1_38611845	325073	4.4	7.1	-0.30	51	N-22
	qDSM3.12	3	74	S3_12907150	S3_12946497	39347	2.9	4.6	0.24	3	Cocodrie
	qDSM10.05	10	26	S10_5341617	S10_5412997	71380	3.0	5.1	0.27	11	Cocodrie
Root Shoot Ratio - IM	*qRSR2.03*	2	17	S2_3854717	S2_4310292	455575	3.4	6.8	-0.02	73	N-22
	qRSR3.34	3	180	S3_34874041	S3_35150742	276701	2.0	4.2	-0.01	53	N-22
	qRSR6.06	6	33	S6_6463372	S6_7403500	940128	2.3	4.8	-0.02	135	N-22

[a] qRL, qSL, qFRM, qFSM, qNT, qDRM, qDSM and qRSR are QTLs for root length, shoot length, fresh root mass, fresh shoot mass, number of tillers, dry root mass, dry shoot mass and root shoot ratio respectively. The number before the decimal indicate chromosome and the number after decimal indicate the physical location of the QTL in mega base pair. [b] LOD is logarithm of odds [c] PVE (%) is percentage of phenotypic variance explained by the QTL

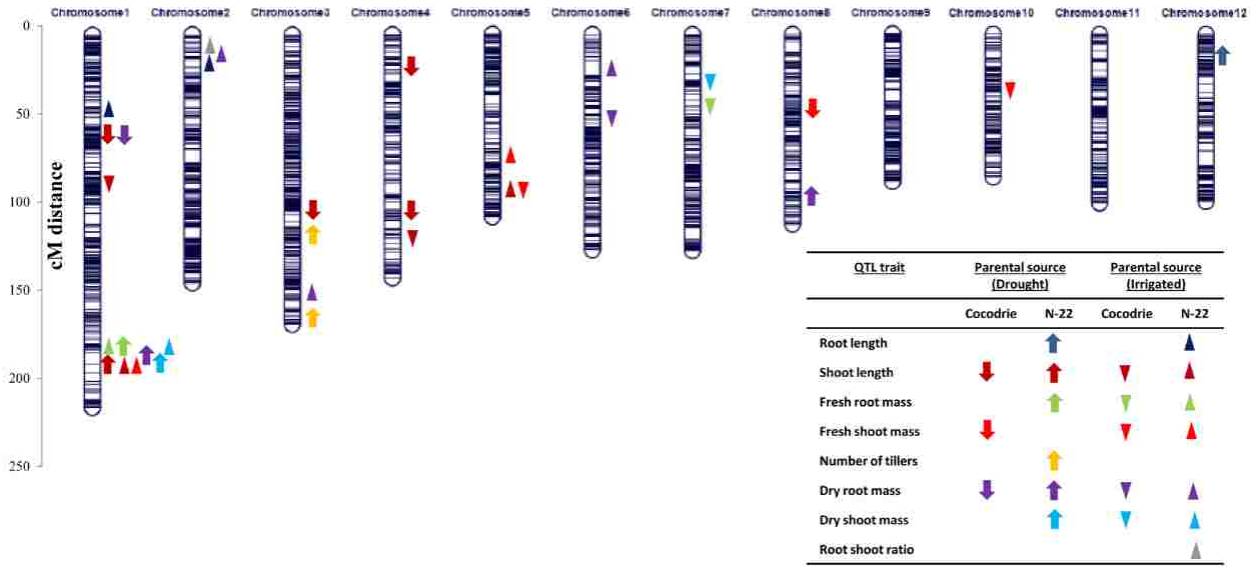

Figure 3.2. Map positions of QTLs for eight root and shoot traits on the genetic linkage map developed in an F_8 RIL population from the cross Cocodrie x N-22. QTLs for these traits under drought stress and irrigated conditions were identified using ICIM mapping procedure. The QTLs expressed under drought stress are indicated by the arrow heads and the QTLs expressed under irrigated condition are indicated by the triangle. The QTL alleles contributing toward increased mean by N-22 are indicated by the arrows pointing upwards and the QTL alleles contributed by Cocodrie are shown by the arrows pointing downwards. Vertical triangles indicated the increasing mean effect for the trait by N-22 allele, whereas the triangle pointing downwards showed the Cocodrie allele responsible for the increasing effect on the trait. Dark regions in the linkage map are the marker saturated regions and the white regions are the gaps between the markers.

these pairs of interacting QTLs. These interacting QTLs were independent of the additive QTLs identified for number of tillers.

3.3.4.6. Dry root mass QTLs

ICIM detected three QTLs for dry root mass under drought stress condition. The *qDRM8.25* was identified in both IM and ICIM mapping procedures. Each of these QTLs accounted for 4-7% of the total variation in phenotype. N-22 alleles were responsible for increasing effect for dry

root mass QTLs *qDRM1.11* and *qDRM1.37*. However, Cocodrie allele increased mean phenotypic value at the *qDRM8.25*. Four QTLs were detected for dry root mass during normal condition. Three of those QTLs (*qDRM2.03, qDRM3.30,* and *qDRM6.06*) were detected by both IM and ICIM methods. There were four pairs of epistatic QTLs identified for dry shoot mass. No additive QTLs co-localized with the identified epistatic QTLs.

3.3.4.7. Dry shoot mass QTLs

One dry shoot mass QTL (*qDSM1.38*) was detected by both IM and ICIM mapping. It accounted for 11% of the total phenotypic variation and the alleles for increased mean effect were contributed by N-22. Two QTLs were detected for dry shoot mass under non-stress condition. These QTLs individually explained 4-11% of the variation in phenotype. Seven pairs of epistatic QTLs were diagnosed for dry shoot mass. The increasing effects in three pairs of these QTLs were contributed by N-22 allele and the mean increasing effects in other four pairs of QTLs were contributed from Cocodrie allele.

3.3.4.8. Root shoot ratio QTLs

One QTL (*qRSR2.03*) was detected by ICIM for root shoot ratio under irrigated condition. It accounted for 8% of the total phenotypic variation and the increasing mean effect was due to the contribution from N-22 allele. Ten pairs of epistatic QTLs were identified for root shoot ratio. The mean increasing effect in six of these QTL pairs were due to N-22 alleles and the increasing effects in four other QTL pairs were due to Cocodrie alleles.

3.3.5. Mapping of segregation distortion loci

Mapping of segregation distortion loci showed nine regions that deviated from 1:1 segregation ratio (Appendix Table B2). Three segregation distortion loci were observed in each of the chromosome 1 and 10, one each on chromosomes 2, 4, and 12. Among the nine, three of

the distortion loci deviated more towards Cocodrie and six loci towards N-22. The size of the segregation distortion loci varied from 25 kb on chromosome 12 to 325 kb on chromosome 10. Segregation distortion loci on chromosome 1 at 95 cM and 109 cM position were highly skewed in favor of N-22. None of the additive and epistatic QTLs identified in our study co-localized with the segregation distortion loci.

3.3.6. Co-localization with previously identified QTLs

To study the robustness of our QTL mapping, we conducted a survey to determine the congruency of the previously reported QTLs with the QTLs identified in this study. Four of our shoot length QTLs co-localized with the previously reported QTLs (Table 3.8). The QTLs, *qSL1.37* and *qSL1.38,* located within the chromosomal region of 37,851,779 - 38,894,388 bp in chromosome 1, co-localized with plant height QTL *qDTH1.1* (Drought tolerant height) (Vikram et al. 2011). Two QTLs at the chromosomal location (26,857,374 - 30,334,896 bp), *qSL4.25* and *qSL4.29,* were similar to *qPH4* detected by Xu et al. (2004). QTL for tiller number *qNT3.28* was located in the same chromosomal region as *qNOT3.2* (Hemamalini et al. 2000). Two QTLs for dry root mass (*qDRM1.38* and *qDRM1.37*) co-localized with the two previously reported dry root mass QTLs in 38 Mb and 37 Mb region of chromosome 1, respectively (Shen et al. 2001; Nguyen et al. 2004). Dry shoot mass QTL (*qDSM1.38)* was located at the same chromosomal region to the previously reported *qDTB1.1* (Vikram et al. 2011). Dry shoot mass QTL *qDSM8.08* co-localized with shoot dry weight QTL reported by Nagata et al. (2002). QTLs for fresh root mass (*qFRM1.22b*) was congruent with the same chromosomal region as previously identified QTL *FRW1c* (Li et al. 2005). The localization of some of our QTLs with the previously reported QTLs showed the consistency and reliability of our mapping procedures.

3.3.7. Gene ontology analysis

Due to high saturation of SNP markers on the linkage map, numbers of genes in QTL confidence intervals were as low as nine genes for *qSL3.24* whereas there were 539 genes for *qFSM8.11*. The total numbers of genes identified in 14 QTLs under drought stress were 1052 with an average of 75 genes per QTL (Additional table 1, Available upon request). The listed candidate genes for each trait were classified for gene ontology groups (Table 3.9; Additional table 2, Available upon request). Among 49 genes for QTL controlling root length, there were 22 genes that were annotated for at least one gene ontology group. There were 10 significant gene ontology terms for the genes controlling root length. Seven gene ontology terms were specific to biological processes and 3 were specific to molecular function. The percentage of genes assigned to at-least one gene ontology term ranged from 45% for root length to 72% for fresh root mass. Among 1052 genes, 534 genes were assigned to at least one gene ontology term. These 534 annotated genes were classified into 362 significant gene ontology terms. There were 199 gene ontology terms identified as biological processes, 142 as molecular function and 21 as cellular component.

3.4. Discussion

Drought is a major abiotic stress which reduces rice productivity in both uplands and rainfed lowlands. Understanding the genetics of drought tolerance is necessary to develop rice varieties that can maintain high yield in drought affected regions. The QTL mapping is a useful tool to identify the chromosomal regions and the genes associated with drought tolerance. The QTL analysis using linkage maps with limited number of markers results in large confidence interval of QTLs, which makes the mapping work less precise and inefficient. GBS is a low depth whole genome sequencing approach, which can be used to generate large number of SNPs between the

lines (Elshire et al. 2011). Using a high-density SNP linkage map facilitated by GBS, QTLs for drought responsive root and shoot traits were identified with narrow confidence intervals that led to identification of candidate genes in this study.

A clear understanding of the genetics of root system under drought stress is necessary to develop the plant ideotype with a better root system. There was a 22% increase in root length in the donor N-22 under drought stress compared to the control environment. Deep root is an important adaptive mechanism of drought tolerant cultivars because it helps the plants to extract water from deep layers of the soil during water stress (Champoux et al. 1995; Zheng et al. 2003; Uga et al. 2013). N-22 was superior to Cocodrie in respect of shoot length, fresh shoot mass, fresh root mass, number of tillers, dry shoot mass, dry root mass, and root-shoot ratio. The reduction in shoot length and shoot mass was greater in N-22 compared to Cocodrie under drought stress. However, the reduction in root mass was more for Cocodrie. The increase in root length, reduction in shoot length, and shoot mass are some of the adaptive features, which should be incorporated for developing drought tolerant rice cultivars (Kamoshita et al. 2002; Uga et al. 2013). Transgressive segregation was observed for all the traits in both directions suggesting contribution of alleles from both parents toward trait manifestation (Ali et al. 2000). High heritability values for all the traits in both the environmental conditions indicated less environmental influence on these traits.

Correlation studies showed that the root length and root biomass were positively correlated to shoot length and shoot biomass, which is in agreement with earlier report (Yadav et al. 1997). This increase in shoot length, shoot biomass, and root length under drought may be due to the increase in water and nutrient uptake capacity of the deeper root system (Yoshida and Hasegawa 1982; Champoux et al. 1995). Shoot length was negatively correlated to number of tillers which

Table 3.8. List of previously reported QTLs co-localized with the QTLs identified in this study

Traits[a]	This study		Previous studies			
	QTL	Position of QTLs (bp)	QTL	Marker interval	Position of QTLs (bp)	References[b]
SL	*qSL1.37*	37089296-37273187	*qDTH1.1*	RM11493-RM431	37851779-38894388	1
	qSL1.38	38023681-38286772				
	qSL4.25	25750452-27194959	*qPH4*	RM241-G102	26857374-30334896	2
	qSL4.29	29179250-29482850				
NT	*qNT3.28*	28513305-28809504	*qNOT3-2*	RZ448-RZ519	28789373-28812372	3
DRM	*qDRM1.38*	38023681-38286772	*TRDW1.1*	RG727-RG109	38280484-38531467	4
	qDRM1.37	37376121-37561874	*Total root weight QTL*	RZ730-RZ801	34937981-40566030	5
DSM	*qDSM1.38*	38023681-38286772	*qDTB1.1*	RM315-RM431	36734135-38894388	1
	qDSM8.08	8971655-9136647	*Shoot Dry weight QTL*	xnpb38-xnpb104	8923052-8924004, 34470620-37713609	6
FRM	*qFRM1.22b*	22796323-22958631	*FRW1c*	RM306-RM5	22796323-22958631	7

[a] SL: Shoot length, NT: Number of tillers, DRM: Dry root mass, DSM: Dry shoot mass, FRM: Fresh root mass

[b] 1: Vikram et al. 2011, 2: Xu et al. 2004, 3: Hemamalini et al. 2000, 4, Nguyen et al. 2004, 5: Shen et al. 2001, 6: Nagata et al. 2002, 7: Li et al. 2005

Table 3.9. Gene ontology (GO) analysis of QTL regions for each drought responsive trait

Traits	Total number of genes	Number of annotated genes[a]	Percentage of genes annotated	Number of significant gene ontology terms[b]			
				Biological Process	Molecular Function	Cellular component	Total
Root Length	49	22	44.9	7	3	0	10
Shoot Length	152	69	45.4	27	18	2	47
Fresh root mass	65	47	72.3	32	14	2	48
Fresh shoot mass	539	257	47.7	61	52	9	122
Number of tillers	96	56	58.3	27	23	4	54
Dry root mass	108	57	52.8	32	22	4	58
Dry shoot mass	43	26	69.5	13	10	0	23
Total	1052	534		199	142	21	362

[a] Total number of genes assigned to at least one GO terms

[b] A significant gene ontology is declared when the p-value of the assigned GO is less than 0.001

could be due to plant's allocation of assimilates in increasing the number of tillers while reducing the plant height (Yoshida and Hasegawa 1982).

The average QTL interval in our study was 0.486 Mb. This narrow confidence interval, compared to previously identified QTLs, would be useful for marker assisted selection and pyramiding of the desired QTLs in high yielding varieties. In addition, QTLs with small confidence intervals can accelerate fine mapping and QTL cloning with less time and effort. Marker assisted pyramiding of drought tolerant QTLs have been done by several researchers (Shamsudin et al. 2016a, b) to incorporate drought tolerance in elite varieties. Grain yield QTL during drought was useful to increase yield under drought stress condition (Swamy et al. 2017). The congruence of drought tolerant QTL *DTY1.1* (Vikram et al. 2011) with the QTL (*qSL1.38*) identified in our study illustrated the importance of secondary agronomic traits in improving grain yield under drought stress conditions.

In this study, QTLs controlling several roots and shoot attributes at the vegetative stage of plant growth under drought stress were identified and compared with those identified under control condition. The root length QTLs under drought stress were different from those identified under irrigated condition. This may be due to the difference in expression of the genes for root length under stress and non-stress environments (Zhang WP et al. 2001). The increase in root length under drought stress may be due to cell wall loosening and expansion of the cell membrane (Zheng et al. 2003). There were 49 genes involved in the *qRL12.04* confidence interval. One of these genes, Brevis radix (BRX) was known to regulate cell proliferation and elongation in the root system in addition to its involvement in brassinosteroid (BR) pathway and in regulation of auxin-responsive genes (Rodrigues et al. 2009). The other two QTLs controlling

root length under non-stress environment were located on chromosomes 1 and 2 and contained 7 and 15 genes, respectively.

Adaptation to drought stress can be achieved by reducing shoot growth. This is evident by reduced plant height under drought stress compared to the control environment in both parents and the RIL population (Table 3.1). Five shoot length QTLs were detected under water stress conditions. The QTL *qSL1.38* was adjacent to the *sd1* locus (38.3 Mb) and contained 43 genes. A tight linkage between *sd1* and drought yield QTL *qDTY1.1* was earlier reported (Vikram et al. 2015). The *Sd1* locus was found to be associated with many underground and above ground traits in rice (Yadav et al. 1997). The *qSL1.38,* co-localized with *qDSM1.38,* explained the positive association between shoot mass and the shoot length. Presence of QTLs in the same genomic region for different traits is expected for highly correlated traits (Ali et al. 2000). The interacting QTLs had a very low contribution to phenotypic variation suggesting minor role of epistasis in controlling the shoot length under drought stress. Genes encoding Universal stress protein (LOC_Os01g19820), Calvin cycle protein (LOC_Os01g19740), and Pentatricopeptide protein (LOC_Os01g19548, LOC_Os01g65840) were present in these QTL regions. The Universal stress protein and Calvin cycle proteins were reported to improve drought tolerance in plants (Sinha et al. 2016; Kwasniewski et al. 2016) whereas the Pentatricopeptide protein was known to regulate the stomatal closure and prevent plants from dehydration (Jiang et al. 2015). The genomic region of the major QTL *qSL4.06* harbored genes such as OsFBX121 - F-box domain containing protein, expressed (LOC_Os04g11890), O-methyltransferase (LOC_Os04g11970), and Glycosyltransferase (LOC_Os04g12010), which were reported to improve abiotic stress tolerance in plants (Lam et al. 2007; Keppler and Showalter 2010; Yan et al. 2011). Drought tolerance in plants can be either due to metabolic regulation or osmoregulation. However, the

molecular mechanism of drought tolerance in rice via osmotic adjustment may be different from that of the avoidance mechanism obtained by developing a deep root system (Zhang J et al. 2001).

Root mass contributes to adaptation and growth of plants under drought stress. The QTL *qFRM1.36* controlling the fresh root mass in rice under drought condition contained 65 genes within its confidence interval. Two important candidate genes present in this QTL region were WRKY (LOC_Os01g62510) and Laccase protein (LOC_Os01g62480, LOC_Os01g62490, LOC_Os01g62600). WRKY gene family is involved in enhancing drought tolerance in rice by reducing cell death, water loss, and electrolyte leakage with increased proline content (Jiang et al. 2016). Laccase gene family, involved in catalytic activity, oxidoreductase, ligase, and hydrolase activity, is known for enhancing tolerance to copper stress in Arabidopsis (Liu et al. 2017).

The decrease in shoot mass during water stress may be one of the adaptive mechanisms in plants that help to accumulate metabolites in roots under water stress (Kamoshita et al. 2002). One QTL on chromosome 8 was identified to control fresh shoot mass under drought stress condition. However, QTLs for fresh shoot mass under irrigated condition was observed on chromosomes 1, 3, 5, and 10. Serine threonine protein kinase gene (LOC_Os08g23290), present in this QTL region, was strongly induced under drought and extreme salinity conditions (Kulik et al. 2011).

The QTLs for number of tillers were mostly concentrated on chromosome 3. Coatomer subunit protein (LOC_Os03g50340, LOC_Os03g50350), heat shock protein (LOC_Os03g61940), and cytochrome P450 (LOC_Os03g061980) were some of the candidate genes present in these QTL regions. The Coatomer protein is involved in cellular process,

localization, and membrane related functions whereas heat shock protein and cytochrome P450 are responsible for the metabolic function and catalytic activity. Cytochrome P450 genes were reported to be drought responsive (Gorantla et al. 2007). Heat shock proteins, responsible for exhibiting universal tolerance to various abiotic stresses like heat, drought, salt, and cold, were expressed in root, stem, leaf, internode, and spikelet during drought stress (Wang et al. 2015)

Dry root mass indicates the amount of dry matter present in the root system, which can be used by the plants during stress condition. Three additive QTLs controlling dry root mass in rice under drought stress were identified by ICIM. The candidate genes present in this QTL region included Cytochrome P450 gene (LOC_Os08g39694), G-patch domain (LOC_Os08g39880), ENT domain (LOC_Os08g39970), and Ty3-gypsy class protein (LOC_Os08g39910). Dry root mass QTLs under well-irrigated conditions differed from those under stress conditions. They were located on chromosomes 2, 3, and 6. In our study, the *qDSM1.37* co-localized with fresh root mass QTL *(qFRM1.37)* under irrigated condition. Both *qDSM1.38* and *qDSM8.08* overlapped the same QTL regions reported in earlier studies (Vikram et al. 2011; Nagata et al. 2002). There was only one QTL *qRSR2.03* for root shoot ratio identified under during irrigated condition and its overlapping with the dry root mass QTL *qDRM2*.03 was corroborated by a strong correlation between these traits. Li et al. (2005) identified a QTL for root shoot ratio in chromosome 6, which co-localized with the QTL for root shoot ratio *qRSR6.06* identified by interval mapping under non-stress environment.

3.5. Conclusions

The high-resolution mapping of QTLs in this study using a high-density SNP linkage map led to the selection of several candidate genes in the QTL regions, which may be involved in some adaptive mechanisms in both root and shoot system leading to enhanced drought tolerance.

The consistent QTLs for drought related traits can be introgressed into adaptive high yielding varietal backgrounds to develop drought tolerant rice varieties. For more precise breeding effort, validation of the role of candidate genes in improving drought tolerance followed by the development gene-based markers should be undertaken.

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Chapter 4. Genetic Analysis of Yield and Agronomic Traits Under Reproductive Stage Drought Stress in Rice Using a High-Resolution Linkage Map

4.1. Introduction

Rice is an important food crop grown all around the world. Due to high water requirement, rice cultivation in the rainfed and water limiting environments is severely affected. Irrigated rice covers 55% of the rice-growing areas and produces 75% of the total production while the remaining is contributed by areas exposed to moisture stresses (CGIAR Science Council 2009). Since rice productivity in irrigated areas has stagnated, efforts should be made to increase rice production in the rainfed ecosystem. Exploitation of natural genetic variation to develop drought tolerant varieties and effective water management practices are some of the strategies to address this challenge.

Drought tolerance in rice is inherently complex involving multiple mechanisms. Phenotyping for drought tolerance should involve primary traits (plant height, root length, and number of tillers), secondary traits (plant water status, leaf rolling, and leaf death) and integrated traits (spikelet fertility, harvest index, and test weight) (Kamoshita et al. 2008). Identification of quantitative trait loci (QTLs) and genes controlling the root traits (Uga et al. 2013; Bhattarai and Subudhi 2018) and various other physiological traits (Zhang et al. 2001, Nguyen et al. 2004) has been done in the past. But due to poor correlation of these traits with yield, there has been little progress in improving rice productivity in drought prone areas. Yield improvement is dependent upon several yield component traits. Progress has been made in developing drought tolerant cultivars by direct selection for yield. However, due to low heritability of yield, it is difficult to make improvement in both yield and drought tolerance simultaneously (Palanog et al. 2014).

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Plant height, spikelet fertility, plant biomass, and harvest index are some associated traits that influence grain yield in rice (Prince et al. 2015). Therefore, QTLs for yield along with other highly heritable yield component traits would be an effective strategy to enhance yield under drought stress.

Efforts have been made to identify drought-tolerant donors and to develop suitable selection criteria for breeding drought-tolerant varieties (Kumar et al. 2014). Several QTLs for drought tolerance have been identified in the past (Bernier et al. 2007; Saikumar et al. 2014; Palanog et al. 2014; Dixit et al. 2014a, 2014b; Prince et al. 2015; Solis et al. 2018). Most of these QTL studies were based on direct selection for yield. QTLs for yield have been identified on chromosomes 1, 2, 3, 6, and 12. The *qtl12.1* was the first yield QTL for drought tolerance at the reproductive stage (Bernier et al. 2007). This QTL has been used in marker-assisted breeding to develop a drought-tolerant version of 'Sabitri', a popular rice variety of Nepal (Dixit et al. 2017b). A large effect reproductive stage drought responsive QTL (*qDTY1.1*) has been identified on chromosome 1 (Ghimire et al. 2012; Venuprasad et al. 2012) with a consistent effect in various genetic backgrounds. A possible linkage between *qDTY1.1* and *sd1* affects the introgression of this yield enhancing QTL to elite varieties (Vikram et al. 2015). Besides this, the drought-tolerant QTLs on chromosome 2 (*qDTY2.1, qDTY2.2,* and *qDTY2.3*) for grain yield were also identified (Palanog et al. 2014). A reduction in flowering duration due to the interaction of *qDTY3.2* with two other QTLs *qDTY1.1* and *qDTY12.1* was observed. Drought tolerant rice varieties could be developed by marker-assisted breeding and QTL pyramiding of drought-tolerant QTLs. An interaction between two or more QTLs was reported to increase grain yield under drought stress (Swamy et al. 2013; Sandhu et al. 2018). Pyramiding drought tolerant QTLs (*qDTY2.2, qDTY3.1,* and *qDTY12.1*) in Malaysian rice variety showed increase in yield

(Shamsudin et al. 2016). Identification of stable large effect QTL across various environments and genetic backgrounds is needed to develop drought-tolerant rice varieties.

QTL based identification and cloning of genes has been helpful for rice improvement (Uga et al. 2013). A limited effort has been made in the past to study drought tolerance in USA rice varieties. In this study, we identified the drought-tolerant QTLs for yield and various yield attributing traits in rice at the reproductive stage drought stress using a recombinant inbred line (RIL) population involving a susceptible southern US rice variety 'Cocodrie'.

4.2. Materials and methods

4.2.1. Development of mapping population

The mapping population comprised of RILs derived from a cross between Cocodrie and N-22. Cocodrie is a high yielding rice variety developed by Louisiana State University Agricultural Center and is sensitive to drought (Linscombe et al. 2000). N-22 is a popular drought tolerant cultivar with low yield potential (Kumar et al. 2014). The F_1s derived from a cross between Cocodrie and N-22 were selfed for six generations and the seeds were advanced by single seed descent method to generate F_7 RILs for drought phenotyping and genotyping.

4.2.2. Phenotyping under drought stress

One hundred eighty-one RILs and two parents (Cocodrie and N-22) were phenotyped for drought tolerance. The experiment was conducted inside the greenhouse of Louisiana State University Agricultural Center during summer 2016. Plants were grown in 2-gallon plastic pots filled with silty clay soil. The pots were placed in a plastic covered concrete bench filled with water. Nutrient solution (0.2%) was prepared using Jack's Professional (20-20-20) (J.R. Peters, Inc.) and was applied every week for the first four weeks of plant growth. Slow release fertilizer 'Osmocote' (4 g) was applied in every pot after 35 d and 55 d of plant growth. Three plants were

grown in each pot. There were three replications and the experiment was conducted in completely randomized design. Two replications were subjected to drought treatment and one set was used as control. The pots were transferred from the irrigated bench to the non-irrigated bench when the plants started to emerge panicle. The plants were left un-irrigated for one week. The degree of drought severity was assessed by observing >50% spikelet sterility in majority of the RILs and some irreversible damage in highly susceptible RILs. After one week, the plants were replaced in the bench filled with water. Irrigation was continued until the plants attained physiological maturity. The control bench was continuously irrigated until harvest. Measurements were taken on three plants from each pot in each replication for morphological and yield parameters. Days to flowering (DTF) was determined as the number of days for emergence of the panicle. Plant height (PH) was measured from the base of the culm to the tip of the plant. Leaf rolling score (LRS) was determined following the standard evaluation system (SES) of rice (IRRI, 2002). Dry matter content (DM) was measured as the ratio of dry plant mass to the fresh plant mass and was expressed in percentage. Grain yield per plant (GY) was measured averaging the gain yield from all drought treated plants in each replication. Yield index (YI) was calculated as the ratio of the plant yield at drought and control condition. Harvest index (HI) was calculated as the percentage ratio of grain yield to the biomass yield under drought stress. Root length, dry root mass, and root shoot ratio were measured under both stress and control environment in the same mapping population in an earlier experiment and the detailed protocol on phenotyping was described in Bhattarai and Subudhi (2018).

4. 2.3. Statistical analysis

Statistical analysis was carried out using SAS 9.3 (SAS Inc. 2011). Frequency distribution graphs were constructed using a pivot table in Microsoft Excel 2010. Mean, standard deviation,

and coefficient of variance were computed using PROC MEANS procedure. Analysis of variance (ANOVA) was estimated using PROC MIXED procedure in SAS, considering the line as fixed effect and replication as random effect. Broad-sense heritability (based on family mean basis) was estimated in SAS using the SAS code (Holland et al. 2003). Pearson correlation coefficients were calculated using PROC CORR procedure. The previously generated data on root traits under both stress and non-stress environments in the same mapping population was used for determining correlation with four yield-related traits (spikelet fertility, grain yield under stress, yield index, and harvest index). Principal component analysis (PCA) was done to understand the relationship among the variables. PCA was done using JMP software (SAS Inc. 2013).

4. 2.4. Linkage mapping and QTL analysis

A total of 4748 SNPs previously generated by genotyping by sequencing (GBS) in Cocodrie x N-22 RILs were used for the construction of linkage map and QTL mapping (Bhattarai and Subudhi 2018). Linkage mapping and QTL analysis were performed using interval mapping (IM) and inclusive composite interval mapping (ICIM) software (Meng et al. 2015). The markers were placed on the linkage map based on the physical map of the reference genome Nipponbare. Kosambi mapping function was used to calculate the genetic distance between the markers (Kosambi 1944). Both additive and epistatic QTLs were identified. The additive QTLs having LOD scores greater than 2 were considered as significant and the epistatic QTLs with LOD scores greater than 4 were considered as significant. The nomenclature of QTLs was done following the procedure described earlier (Bhattarai and Subudhi 2018). For example, a QTL for days to flowering in chromosome 3 and 1Mb region is written as *qDTF3.01*. A positive additive

effect represented contribution of Cocodrie allele toward increased trait mean and a negative additive effect indicated N-22 allele responsible for increasing the trait mean.

4.2.5. Gene ontology and annotation

The QTL intervals were determined by the physical position of the SNP markers flanking the respective QTLs. All the genes present in each QTL interval was retrieved from the MSU rice genome annotation project database (http://rice.plantbiology.msu.edu/). Trait-wise gene ontology annotation was done using agriGo gene ontology analysis toolkit (Tian et al. 2017). Singular enrichment analysis (SEA) tool in agriGO was used to find the gene ontology term. The suggested locus ID from Gramene database (http://www.gramene.org/) was used as a background for the analysis. The significance of the GO terms was tested using the hypergeomatric statistical test method with a significance threshold of 0.05. The significant gene ontology terms for each trait were classified for biological process, molecular function, and cellular component.

4.2.6. Differential expression of the genes

The data on differentially expressed genes in N-22 under both stress and non-stress conditions were retrieved from Shankar et al. (2016) and the expression of candidate genes within the QTL flanking markers were analyzed. The expression details of genes within the important QTL regions (PVE > 7% or confidence interval < 100kb) were manually checked to determine the differentially expressed genes. The genes present within the QTL intervals and also significantly differentially expressed based on the transcriptome data (Shankar et al. 2016) were labeled as the candidate genes. The plant parts in which these candidate genes were expressed were identified using Affymetrix gene chip data (NCBI database: GSE24048, GSE 26280, GSE25176, GSE41647) available in Genevestigator (Hruz et al. 2008).

4.3. Results

4.3.1. Performance of parents and RILs under drought

The parents showed significant differences for all traits except for leaf rolling score and plant dry matter content (Table 4.1). N-22 showed early flowering (72 d) compared to Cocodrie (78 d). The spikelet fertility and grain yield for N-22 were higher (58% and 11.3 g/plant) than that of Cocodrie (34% and 2.9 g/plant), respectively. A higher yield index was observed for N-22 (0.30). A low harvest index was observed for both the parents; however, it was numerically higher in N-22 (18%) compared to that of Cocodrie (5%). A moderate coefficient of variation was observed for all traits except for harvest index (97%), indicating a wide variation among the RILs. The heritability was high for days to flowering and plant height. A moderate heritability was observed for all other traits except spikelet fertility which had a low heritability. The frequency distribution showed a nearly normal distribution for all traits (Figure 4.1). The RILs showed transgressive segregation on both sides. Analysis of variance showed significant differences among the RILs for all traits (Appendix Table C1).

Table 4.1. Various statistical parameters for yield and yield-related traits under reproductive stage drought stress in Cocodrie × N-22 RIL population

Trait	Parents		RILs			
	N-22	Cocodrie	Mean	SD[a]	CV[b]	Heritability[c]
Days to flowering	72	78**	76.3	5.4	24.5	0.95
Plant height (cm)	93.7	77.7*	97.9	23.8	24.3	0.94
Leaf rolling score	4.0	5.0ns	5.3	1.6	29.9	0.72
Plant dry matter content (%)	49.4	50.2ns	40.2	13.0	32.5	0.66
Spikelet fertility (%)	57.9	33.9*	39.3	25.5	64.8	0.36
Grain yield under stress (g/plant)	11.3	2.9*	8.1	5.4	66.5	0.62
Yield index	0.3	0.1*	0.4	0.3	65.3	0.63
Harvest index (%)	17.7	5.3*	13.6	13.2	97.3	0.78

*,**Difference between the mean value of Cocodrie and N-22 significant at 5% and 1% level of probability, respectively; nsNon-significant
[a]Standard deviation
[b]Coefficient of variation (%)
[c]Heritability (broad sense)

4.3.2. Trait associations

Most of the traits showed significant correlations with each other except plant dry matter content (Table 4.2). Plant dry matter content was significantly and negatively correlated only to plant height and yield. Days to flowering, plant height, and leaf rolling score were negatively correlated to spikelet fertility, grain yield, yield index, and harvest index. The grain yield was highly correlated to spikelet fertility, yield index, and harvest index. Leaf rolling score was negatively and significantly correlated with grain yield, spikelet fertility, yield index, and harvest index. No significant correlations were observed between root parameters and yield under drought stress (Appendix Table C2.). However, a negative and significant correlation was observed between root length and spikelet fertility under control condition.

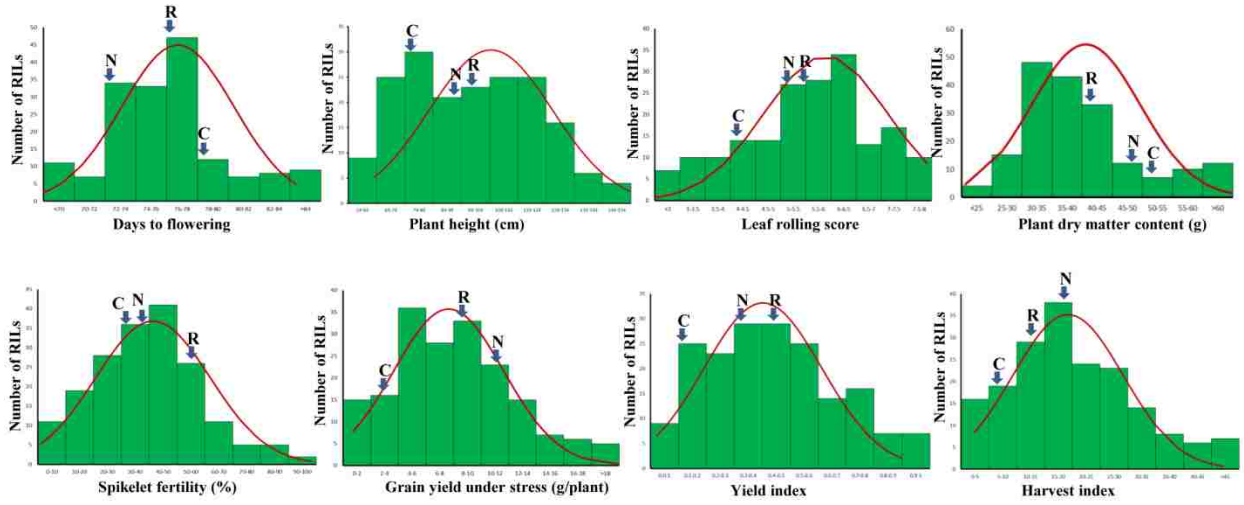

Figure 4.1. Frequency distribution for various yield and yield related traits in Cocodrie x N-22 RIL population under reproductive stage drought stress. The traits include days to flowering, plant height, plant dry matter content, spikelet fertility, grain yield under stress, yield index, and harvest index. The parental and RIL mean are represented by arrows pointing downwards. C, N, and R indicate Cocodrie, N-22, and RIL means, respectively.

Table 4.2. Correlation coefficients among various yield and yield-related traits under reproductive stage drought stress in Cocodrie × N-22 RIL population

Trait[$]	DTF	PH	LRS	DM	SF	GY	YI	HI
DTF	1	0.24**	0.21**	0.06 ns	-0.17*	-0.23**	-0.20*	-0.17*
PH		1	0.47**	-0.16*	-0.31**	-0.09 ns	-0.11 ns	-0.27**
LRS			1	-0.05 ns	-0.43**	-0.24**	-0.29**	-0.29**
DM				1	0.07ns	-0.18*	-0.09 ns	0.02 ns
SF					1	0.50**	0.43**	0.44**
GY						1	0.67**	0.73**
YI							1	0.56**
HI								1

[$]DTF, Days to Flowering; PH, Plant height (cm); LRS, Leaf rolling score; DM, Plant dry matter content (%); SF, Spikelet fertility (%); GY, Grain yield under stress (g); YI, Yield index; HI, Harvest index (%) *,**Significant correlation at 5% and 1% level of probability, respectively
nsNon significant

4.3.3. Principal component analysis

The principal component analysis, conducted for all the traits, projected three important principal components (Appendix Table C3.). The first and the second principal components explained 39% and 17% of the total variation. The three principal components explained a cumulative of 69% of the total variation. The first principal component was associated with yield-related traits such as spikelet fertility, yield under drought stress, yield index, and harvest index (Appendix Table C4., Figure 4.2). The second principal component accounted for shoot related traits like plant height, leaf rolling score, and plant dry matter content. The third principal component was related to days to flowering. There were three component traits in our study that included yield component, shoot component, and days to flowering.

4.3.4. QTL analysis

QTL analysis was based on 4748 GBS-based SNP markers (Bhattarai and Subudhi 2018). Interval mapping (IM) and Inclusive composite interval mapping (ICIM) detected 31 and 21 QTLs for eight traits, respectively (Table 4.3, Appendix Table C5.). The phenotypic variance explained by these QTLs ranged from 2.4-47.4% and 2.8-24.7% in ICIM and IM analyses,

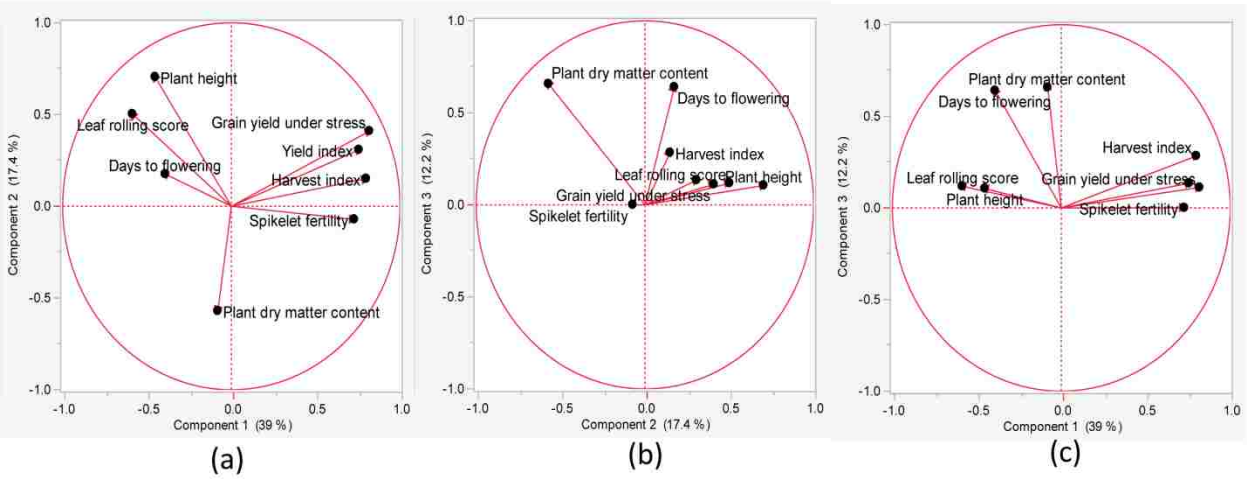

Figure 4.2. Principal component (PC) analysis of eight yield and yield related traits in rice. Each trait is indicated by one projected arrow. The lines indicate the direction and magnitude of the variable's contribution to the principal component. The principal components are shown in the axis and the variance contributed by each principal component is indicted inside the parentheses: (a) component analysis between PC1 and PC2, (b) component analysis between PC2 and PC3, and (c) component analysis between PC1 and PC3.

respectively. Although ICIM detected QTLs on eight different rice chromosomes, majority of them (8) were located on chromosome 1 (Figure 4.3). Inclusive composite interval mapping detected two QTLs (*qDTF3.01* and *qDTF11.08*) for days to flowering with phenotypic variances of 13% and 7%, respectively. The favorable alleles for both QTLs came from Cocodrie. Interval mapping detected eight QTLs for days to flowering. Both ICIM and IM detected *qDTF3.01*. Interval mapping detected five QTLs controlling days to flowering on chromosome 3. Five plant height QTLs were detected by both ICIM and IM method. The QTLs for plant height were clustered on chromosome 1. *qPH1.38* was a major effect QTL with a LOD score of 35 and a phenotypic variance of 47%. The favorable allele for this QTL was contributed by N-22. The other QTLs detected for plant height under stress were on chromosomes 3, 5, and 9.

Table 4.3. List of additive QTLs for yield and yield-related traits identified using ICIM in Cocodrie × N-22 RIL population under reproductive stage drought stress

Traits[a]	QTL[b]	Chr[c]	Genetic Pos. (cM)	Physical Pos. (Mb)	Left Marker	Right Marker	LOD[d]	PVE (%)[e]	Additive effect	Favorable parental allele
DTF	*qDTF3.01*	3	5	1.11-1.50	S3_1110340	S3_1497144	4.40	12.5	1.60	Cocodrie
	qDTF11.08	11	44	8.95-9.00	S11_8950871	S11_9004531	2.51	6.9	1.20	Cocodrie
PH	*qPH1.07*	1	39	7.06-7.08	S1_7055941	S1_7080781	2.72	2.4	3.64	Cocodrie
	qPH1.38	1	193	38.29-38.61	S1_38286772	S1_38611845	34.77	47.4	-16.31	N-22
	qPH3.32	3	207	32.85-33.29	S3_32853301	S3_33287194	2.75	2.5	-3.73	N-22
	qPH5.24	5	98	24.89-25.27	S5_24888719	S5_25271367	4.17	3.8	-4.60	N-22
	qPH9.14	9	57	14.85-15.32	S9_14854433	S9_15323072	4.50	4.5	5.02	Cocodrie
LRS	*qLRS1.37*	1	186	37.27-37.37	S1_37273187	S1_37368297	8.70	18.1	-0.57	N-22
	qLRS7.07	7	43	7.18-8.03	S7_7182433	S7_8031540	3.48	6.6	0.35	Cocodrie
	qLRS12.17	12	67	17.79-19.26	S12_17786116	S12_19257062	3.08	6.2	-0.33	N-22
DM	*qDM1.07*	1	39	7.06-7.08	S1_7055941	S1_7080781	2.53	5.3	2.65	Cocodrie
	qDM3.33	3	207	33.00-33.07	S3_33000563	S3_33074353	2.97	6.7	3.00	Cocodrie
SF	*qSF1.38*	1	193	38.29-38.61	S1_38286772	S1_38611845	3.70	7.7	-5.35	N-22
	qSF6.23	6	98	23.93-24.29	S6_23928806	S6_24288517	2.17	4.1	3.87	Cocodrie
	qSF7.0.4	7	0	0.43-0.55	S7_425945	S7_553805	3.92	7.8	-5.39	N-22
	qSF11.19	11	71	19.37-19.70	S11_19374580	S11_19696359	2.79	5.8	4.67	Cocodrie
GY	*qGY1.42*	1	241	42.93-42.97	S1_42928362	S1_42966746	2.10	6.1	-1.03	N-22
YI	*qYI1.42*	1	242	42.98-43.07	S1_42981696	S1_43065133	2.24	5.0	-0.06	N-22
	qYI12.03	12	29	39.37-40.53	S12_3937337	S12_4053360	2.36	5.3	-0.06	N-22
HI	*qHI1.37*	1	188	37.56-37.74	S1_37561874	S1_37740707	3.59	7.0	3.63	Cocodrie
	qHI6.25	6	114	25.15-25.72	S6_25146090	S6_25717883	2.02	3.7	2.65	Cocodrie

[a]DTF, Days to Flowering; PH, Plant height (cm); LRS, Leaf rolling score; DM, Plant dry matter content (%); SF, Spikelet fertility (%); GY, Grain yield under stress (g); YI, Yield index; HI, Harvest index (%)

[b]The name of the QTL. 'q' followed by the abbreviation of the trait, followed by the chromosome number, one decimal place and the physical location of the QTL in mega base pair [c]Chromosome number in which the QTL is present. [d]Logarithm of odds value for the individual QTL [e]Phenotypic variance explained by the QTL expressed in percentage

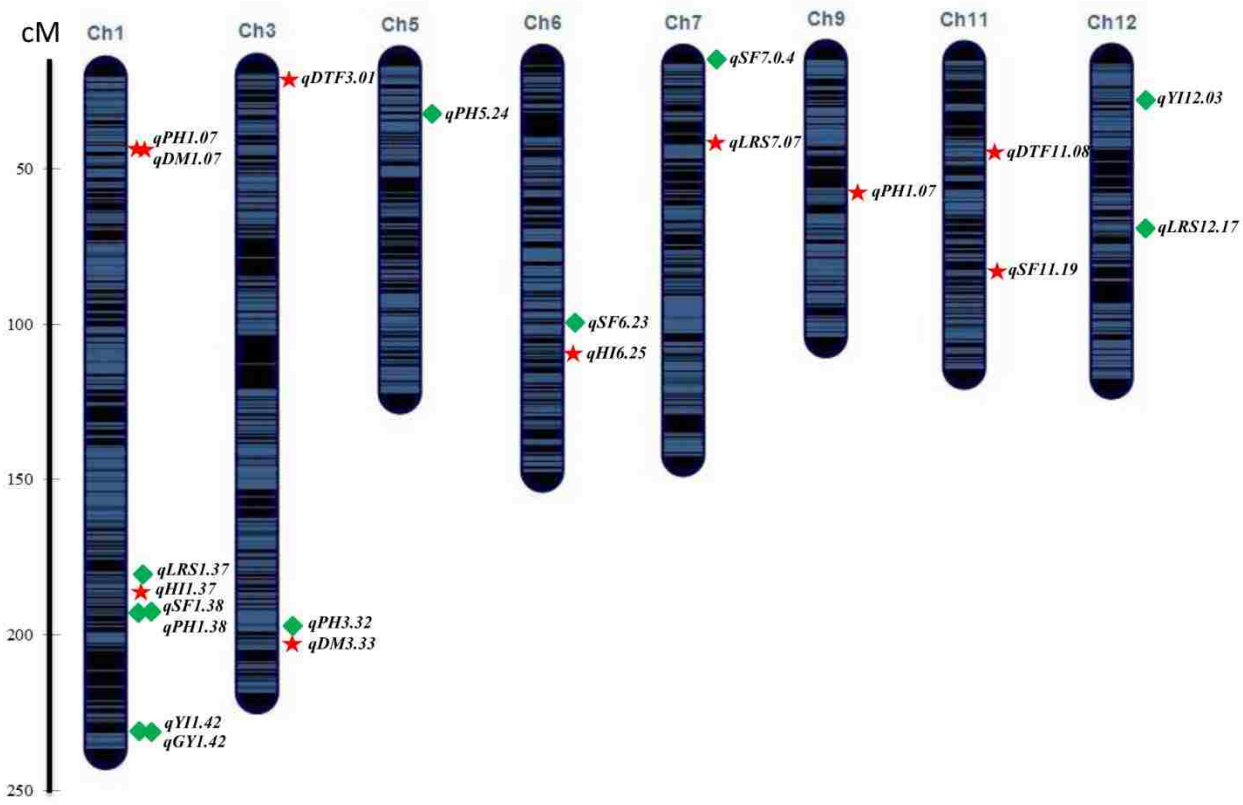

Figure 4.3. Genetic map showing the positions of QTLs for eight yield and yield related traits in Cocodrie x N-22 RIL population. The QTLs for these traits under reproductive stage drought stress were identified by ICIM. The QTLs represented by star and square indicated that the alleles for these QTLs are contributed by Cocodrie and N-22, respectively. The saturation of the markers in the linkage map is indicated by the dark lines. The vertical line on the left show the scale for genetic length of chromosomes.

Inclusive composite interval mapping discovered three QTLs for leaf rolling score (*qLRS1.37*, *qLRS7.07*, and *qLRS12.17*). Both ICIM and IM detected *qLRS1.37* and *qLRS7.07*. The *qLRS1.37* accounted for 18% of the phenotypic variance and the increasing mean effect for this QTL came from N-22 allele. The phenotypic variances contributed by *qLRS7.07* and *qLRS12.17* were 7% and 6%, respectively. Two QTLs for plant dry matter content (*qDM1.07* and *qDM3.33*) were detected by ICIM; the alleles for increasing mean effect in both these QTLs were contributed by Cocodrie. The QTLs, *qDM1.07* and *qDM3.33*, overlapped with the plant height QTLs *qPH1.07* and *qPH1.32*, respectively.

Four QTLs controlling the spikelet fertility were identified by ICIM procedure. Both IM and ICIM detected *qSF1.38* and *qSF7.04*. The *qSF1.38* overlapped with the plant height QTL and explained 8% of the phenotypic variance. There were two other spikelet fertility QTLs detected by interval mapping at 39 Mb and 40 Mb positions on chromosome 1.

Both the mapping procedures discovered one QTL controlling the grain yield under stress. The grain yield QTL, *qGY1.42* was at 42 Mb position on chromosome 1. It contributed only 6% of the phenotypic variance and the mean increasing effect in this QTL was due to the N-22 allele. Two QTLs for yield index (*qYI1.42* and *qYI12.03*) were mapped by both IM and ICIM; the alleles for increasing mean effect for both QTLs came from N-22. Each QTL explained 5% of the phenotypic variance. ICIM and IM identified two (*qHI1.37* and *qHI6.25*) and three (*qHI1.37, qHI1.42* and *qHI3.24*) QTLs for harvest index, respectively. *qYI1.37* accounted for 7% of the phenotypic variance. The increasing mean effects for both *qHI1.37* and *qHI6.25* were due to Cocodrie alleles.

Nine epistatic QTLs with LOD values greater than 4.0 were identified for six traits by ICIM procedure (Appendix Table C6, Appendix Figure C1). Among these QTLs, two were intra-chromosomal epistatic QTLs. These two intra-chromosomal QTLs were discovered on chromosomes 3 and 4; and were responsible for controlling plant height and plant dry matter content, respectively. Another epistatic QTL combination was for spikelet fertility on chromosomes 4 and 8. Three grain yield epistatic QTLs were found to be interacting in chromosomes 2, 3, 4, and 8. One epistatic QTL each for yield index and harvest index was observed. The epistatic QTLs did not overlap with any of the additive QTLs detected.

4.3.5. Gene annotation and ontology study

A total of 989 genes were located within 21 QTL regions identified in this study (Additional table 4.3, Available upon request). The average number of genes per QTL was 45 (Table 4.4). Leaf rolling score had 357 genes within 3 QTL regions. However, the grain yield QTL harbored only four genes. Among all identified genes, 44% were annotated with agriGO. Gene ontology (GO) study revealed 463 GO terms for all eight traits. One hundred twenty-seven GO terms each were identified for plant height and leaf rolling score. Two GO terms were identified for one annotated gene present in grain yield QTL. The number of significant GO terms was quite small with only 64 being significant at the threshold value of 0.05. All significant GO terms for all traits were listed in Appendix Table C7. There were 30 significant GO terms for days to flowering. Most of these GO terms were categorized as biological processes and were responsible for the regulation of metabolic processes. The genes for two yield-related traits, spikelet fertility and harvest index, contained 15 and 3 significant GO terms, respectively. The GO terms for these traits included regulation of various biological pathways and enzyme activities.

4.3.6. Differential expression of genes within the QTL regions

There were 8 significantly up-regulated genes and 2 significantly down-regulated genes ($p < 0.05$) in 6 important QTL regions under drought stress (Table 4.5). The gene regulating fatty acid hydroxylase (*LOC_Os03g03370*) was up-regulated and may be responsible for days to flowering. The large effect QTL *qPH1.38*, and *qSF1.38* contained two genes (mitochondrion protein, and no apical meristem protein) that were differentially expressed under drought stress. Four genes were differentially expressed for the QTLs controlling leaf rolling score. MYB family transcription factor and no apical meristem protein were the notable up-regulated genes in

these QTL regions. The *qSF7.0.4* controlling the spikelet fertility had one major up-regulated potassium channel protein gene. Two genes (*LOC_Os01g64920* and *LOC_Os0164960*) in harvest index QTL (*qHI1.37*) were down-regulated during drought stress. These genes were responsible for nuclear matrix protein 1 and chlorophyll A-B binding protein, respectively.

The above ten differentially expressed genes were expressed in five different parts of the rice plant. *LOC_Os01g64350* was highly expressed in panicle. *LOC_Os01g64960*, *LOC_Os01g66120*, *LOC_Os07g01810*, and *LOC_Os03g03370* were expressed in the shoot parts i.e. seedling, flag leaf, and leaf, whereas *LOC_Os01g66120*, and *LOC_Os01g64960* were highly expressed in roots under drought stress (Appendix Figure C2).

4.4. Discussion

Drought is a major issue in rainfed and upland rice producing ecosystem around the world. Yield reduction is pronounced in areas experiencing drought stress (Palanog et al. 2014; Shamsudin et al. 2016). Using a dense linkage map, drought responsive QTLs were identified in narrow confidence intervals followed by identification of candidate genes within the QTL regions. This helped to narrow down the number of drought responsive genes. Although the yield potential of Cocodrie was much higher in well-watered condition compared to N-22, it showed significantly lower yield under drought stress. Yield index showed the potential of plants to produce grains under moisture stress compared to control condition. A higher yield index indicates better performance under stress condition. N-22 showed a higher yield index suggesting its better performance under drought compared to Cocodrie. High heritabilities for days to flowering and plant height indicated that these traits were least influenced by environmental conditions. However, higher environmental influence was observed for spikelet

Table 4.4. Gene ontology (GO) analysis of the genes present in QTL interval for each trait

Trait[a]	Number of QTLs[b]	Number of genes[c]	Number of genes/QTL	Number of annotated genes[d]	% of genes annotated	Number of GO terms	Number of significant GO terms[e]	% of significant GO terms
DTF	2	58	29	28	48.2	56	30	53.6
PH	5	238	47.6	120	50.4	127	9	7.1
LRS	3	357	119	143	40.0	127	7	5.5
DM	2	17	8.5	12	70.6	3	0	0
SF	5	173	34.6	64	37.0	90	15	16.7
GY	1	4	4	1	25	2	0	0
YI	2	29	14.5	13	44.8	12	0	0
HI	2	113	56.5	49	43.4	46	3	6.5
Total	22	989	45	430	43.5	463	64	13.82

[a]DTF, Days to Flowering; PH, Plant height (cm); LRS, Leaf rolling score; DM, Plant dry matter content (%); SF, Spikelet fertility (%); GY, Grain yield under stress (g); YI, Yield index; HI, Harvest index (%)
[b]Total number of QTLs identified for each trait
[c]Total number of genes present within all the QTL region identified for the specific trait
[d]Number of genes which are assigned at-least one gene ontology terms
[e]GO terms with the p-value less than 0.05

Table 4.5. List of differentially expressed genes present within the identified important drought responsive QTL regions in N-22

Trait	*QTL*	Differentially expressed gene	Regulation[a]	Gene function
Days to flowering	*qDTF3.01*	*LOC_Os03g03370*	Up	Fatty acid hydroxylase
Plant height	*qPH1.38*	*LOC_Os01g66120*	Up	No apical meristem protein
		LOC_Os01g66240	Up	Mitochondrion protein
Leaf rolling score	*qLRS1.37*	*LOC_Os01g64310*	Up	No apical meristem protein
		LOC_Os01g64340	Up	Expressed protein
		LOC_Os01g64350	Up	Expressed protein
		LOC_Os01g64360	Up	MYB family
Spikelet fertility	*qSF1.38*	*LOC_Os01g66120*	Up	No apical meristem protein
		LOC_Os01g66240	Up	Mitochondrion protein
	qSF7.0.4	*LOC_Os07g01810*	Up	Potassium channel protein
Harvest index	*qHI1.37*	*LOC_Os01g64920*	Down	Nuclear matrix protein 1
		LOC_Os01g64960	Down	Chlorophyll A-B binding protein

[a]UP: up-regulation of genes, Down: down-regulation of genes

fertility. A moderate to low heritability for yield was observed in the present study which agrees in earlier studies (Palanog et al. 2014, Solis et al. 2018).

Correlations among the traits help to select the secondary traits for use in crop improvement. The negative correlation of plant shoot traits with yield-related traits suggested that the early flowering and dwarf plants may increase yield (Khowaja et al. 2009; Dixit et al. 2014; Palanog et al. 2014). A high correlation of grain yield with spikelet fertility, harvest index, and yield index under drought stress showed that these integrated traits can be used to improve grain yield in rice (Prince et al. 2015). Similarly, leaf rolling score could also be used as an early selection criterion to increase yield in plant breeding programs. Yield was not affected by length and mass of the root which was evident from the correlation between root traits and the yield traits under water stress. The increase in root length and root mass during water stress increased the plant's

tolerance to drought (Bhattarai and Subudhi 2018). However, this increase might not always be responsible for increase in yield (Khowaja et al. 2009).

In this study, drought responsive QTLs were identified on all chromosomes except chromosomes 2, 4, and 8. The region on chromosome 1 (37.2- 38.6 Mb) was identified as QTL hotspot with four QTLs within 1.4Mb region. This region harbored many QTLs with a large impact on grain yield and plant height (Dixit et al. 2014). We identified 2 QTLs for days to flowering on chromosomes 3 and 11. *qDTF3.01* co-localized with the grain yield QTL *qDTY3.2* (Vikram et al. 2016) and the flowering locus *Hd9* (Lin et al. 2002). This observation indicated that the increase in yield under drought stress may be due to early flowering nature of the plant which helped them to escape the drought stress. This locus was not only responsible for increasing grain yield under drought stress but was also responsible for maintaining cooler canopy temperature which might contribute towards increasing the grain yield (Saikumar et al. 2014).

A reduction in plant height under drought stress was observed in rice (Saikumar et al. 2014). Two QTLs for plant height (*qPH1.07* and *qPH1.38*) on chromosome 1 co-localized with the QTLs for plant dry matter content and spikelet fertility. The major effect QTL *qPH1.38* co-localized with the previously identified large effect yield QTL *qDTY1.1* (Vikram et al. 2011, Venuprasad et al. 2012). Although this QTL was tightly linked to *sd1* locus, it was not responsible for increasing yield under drought stress (Khowaja et al. 2009; Vikram et al 2015). Our QTL mapping approach using a saturated genetic map followed by scanning of the differentially expressed genes in the *qPH1.38* region helped us to exclude the involvement of *sd1* in imparting drought tolerance (Vikram et al. 2015). Based on the gene expression data of N-22 under drought stress, two genes, no apical meristem protein (*NAC*) (*LOC_Os01g66120)*,

mitochondrion protein (*LOC_Os01g66240*) were identified as possible candidates for future investigation (Table 4.5). The gene encoding NAC protein was highly expressed during drought and salinity (Dixit et al. 2014; Hu et al. 2015; Swamy et al. 2017). Another QTL *qPH9.14* co-localized with the grain yield QTL *qDTY9.1*, which was expressed under both moderate and severe drought stress (Swamy et al. 2013) and was reported to control the spikelet fertility under drought (Yue et al. 2005). The QTLs identified for both shoots and panicle traits indicated that the same set of genes can be expressed in different parts of the plant and could affect different traits.

Leaf rolling score was a good early indicator of plants' tolerance to drought. It was possible that the same QTLs may be expressed to govern both shoot traits and yield (Prince et al. 2015). Therefore, selection for yield during the drought condition could be made by observing the leaf rolling in rice. We detected a QTL that controlled both leaf rolling score (*qLRS1.37*) and harvest index (*qHI1.37)*. This region (37.2- 37.3Mb) was identified downstream of the *sd1* locus (38.3Mb). The QTL hotspot for grain yield and many other agronomic traits was previously identified (Venuprasad et al. 2012; Kumar et al. 2014). The QTL for leaf rolling score (*qLRS12.17)* was the same QTL that was previously identified as a large-effect QTL for grain yield (*qtl12.1*) under drought stress (Bernier et al. 2007). This QTL was responsible for increasing the plant water uptake by 7% under water-limited condition (Bernier et al. 2009). It had been incorporated into a popular rice variety 'Sabitri' from Nepal to improve its drought tolerance (Dixit et al. 2017b).

Increase in grain yield is one of the major objectives of any plant breeding program. In this study, QTLs for spikelet fertility, grain yield, yield index, and harvest index were identified. The QTL for spikelet fertility *qSF1.38* co-localized with the previously identified grain yield QTL

qDTY1.1 (Vikram et al. 2015). Co-localization of QTLs was earlier reported (Prince et al. 2015). Three new QTLs on chromosomes 6, 7, and 11 were identified for spikelet fertility. One novel QTL affecting the grain yield under drought stress was identified at 42 Mb position on chromosome 1. This QTL was also responsible for yield index. The QTL spread within 38 Kb genomic region and harbored only 5 genes. A new QTL for yield index was located upstream of *DTY12.1* on chromosome 12. There were two QTLs for harvest index in this study. The *qHI1.37* was present upstream of *sd1* locus and another QTL *qHI6.25* co-localized with an earlier identified QTL *qDTY6.1* (Dixit et al. 2014). This QTL pyramided with *SUB1* provided tolerance to both drought and submergence in rice (Dixit et al. 2017a). Therefore, the QTLs identified in this study offer opportunity to develop new drought-tolerant varieties using marker-assisted breeding and QTL pyramiding.

Both pleiotropic and epistatic interactions were involved in governing drought tolerance in rice (Solis et al. 2018). Root related QTLs identified in previous mapping experiment using the same mapping population (Bhattarai and Subudhi 2018) did not co-localize with the yield QTLs in this study. The absence of correlation between root traits and yield components was in agreement with the observation of Khowaja et al. (2009). However, there were reports regarding involvement of drought tolerant QTLs in improving yield which might be due to increased water uptake and root characteristics (Bernier et al. 2009; Catolos et al. 2017). The contrasting results may be due to the difference in genetic backgrounds and the environments.

4.5. Conclusions

The success of any QTL mapping lies in identifying large effect and stable QTLs in a narrow confidence interval. Using a high-density SNP map, three major QTLs (*qDTF3.1, qPH1.38* and *qLRS1.37*) were identified. Majority of drought tolerant QTLs were identified on chromosome 1.

Identification of the differentially expressed genes within the QTL region helped to reduce the number of candidate genes associated with drought tolerance. Ten differentially expressed genes in 6 QTL regions were identified as potential candidate genes for validation in future before incorporating them into breeding program to develop drought tolerant rice cultivars.

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Chapter 5. Conclusions

Rice is a major crop grown all over the world. It is mostly grown in lowland areas with adequate water supply. Upland rice cultivation is also in practice but not common due to low yield. Global climate change is affecting the agricultural production. Availability of water for agricultural purpose is declining gradually. With all these constraints, rice cultivation is becoming a challenge in all rice growing areas of the world. Therefore, the development of high yielding rice genotypes with reduced water requirement is necessary.

Exploration of the available germplasm resources is the first step in every successful breeding program. Although low genetic diversity in USA rice germplasm is well documented, our drought tolerance screening experiment revealed several US genotypes such as Jes, Leah, Roy J, Jazzman, Madison, Lacassine, and Glutinous zenith as drought tolerant. Principal component analysis showed a high degree of variation for tolerance to drought in USA rice germplasm. The variation in drought tolerance among rice genotypes was not dependent on the state which released them except for rice genotypes from California. Most of the California genotypes were separated from the rice genotypes from other states and were more susceptible to drought. Our study concluded that plant biomass, harvest index, and number of tillers were important traits for drought tolerance screening in rice. The average PIC of the USA rice collection was 0.33 which was less compared to the average PIC of the global rice collection. Further, population structure analysis revealed that the USA rice genotypes can be differentiated based on the US states of origin. The rice varieties from Louisiana were separated from the rice varieties from the other states of USA. Texas and Arkansas rice varieties were closely related, whereas the rice varieties from California formed a distinct group. The markers, RM523 and RM570, were associated with grain yield in rice under drought stress. Similarly, a new marker

RM351 on chromosome 7 was linked with spikelet fertility, grain yield, and harvest index with a phenotypic variance of 7%. These markers will be useful for direct selection of yield under drought.

Root plays a vital role in drought tolerance capacity of rice. Drought tolerant genotypes tend to increase the root length under drought stress. There was 22% increase in root length in the donor N-22 under drought stress compared to the control environment. A new QTL (*qRL12.04)* controlling the root length under drought stress was identified in our study. Two more QTLs for root length, *qRL2.04* and *qRL1.08,* were identified under non-stress condition. Some large effect QTLs for shoot length (*qSL1.38, qSL4.06*) and dry shoot mass (*qDSM1.38*) were also identified. The high-resolution QTL mapping helped in identifying the candidate genes in the QTL regions.

Reproductive stage drought is most detrimental for rice cultivation. It drastically reduces grain yield. Identification of QTLs for drought tolerance at the reproductive stage is needed to stabilize rice productivity in drought prone areas. The negative correlation of leaf rolling score with spikelet fertility, grain yield, yield index, and harvest index, suggested the utility of LRS as an early selection criterion for yield traits. Two major QTLs for days to flowering (*qDTF3.01* and *qDTF11.08)* with a phenotypic variance of 13% and 7%, respectively, were identified. Majority of drought tolerant QTLs were identified on chromosome 1. One new QTL for grain yield under drought (*qGY1.42)* in chromosome 1 with a phenotypic variance of 6% was mapped. Three major QTLs (*qDTF3.1, qPH1.38* and *qLRS1.37*) for drought tolerance at flowering stage were mapped on the high-density SNP map. Identification of differentially expressed genes within the QTL regions helped to identify the candidate genes associated with the traits. Ten differentially expressed genes in 6 QTL regions were identified as potential candidates for

drought tolerance. Validation of the role of these candidate genes for drought tolerance is necessary before incorporating them in any plant breeding program.

In summary, the USA rice collection contained some drought tolerant genotypes based on our evaluation in greenhouse condition. These genotypes can be used as donors in drought breeding programs. The genetic diversity of USA rice collection is very low. Therefore, introduction of new genotypes and the hybridization with the exotic germplasms is necessary to increase genetic diversity in US rice germplasm. Although large number of QTLs were identified for various root traits, agronomic traits, and yield attributes in this study, the large effect QTLs should be given immediate attention for validation and incorporation into elite varieties to enhance drought tolerance. Pyramiding of QTLs associated with root traits and above ground traits may lead to development drought tolerant rice varieties.

Appendix A. Supplementary Information for Chapter 2

Table A1. List of rice genotypes used in the experiment, their source of origin, subtype and the sub-group as classified by structure software

S. N	Genotype	Source[a]	Sub-type[b]	Sub-group[c]	S. N	Genotype	Source	Sub-type	Sub-group
1	Hasawi	International	*Indica*	NG	36	Jazzman	Louisiana	*Japonica*	AD
2	Cheriviruppu	International	*Indica*	SG3	37	Neptune	Louisiana	*Japonica*	SG4
3	Pokkali	International	*Indica*	SG3	38	Caffey	Louisiana	*Japonica*	SG4
4	Nona Bokra	International	*Japonica*	NG	39	Templeton	Louisiana	*Japonica*	SG6
5	Capsule	International	*Indica*	NG	40	Taggert	Louisiana	*Japonica*	SG6
6	FL478	International	*Japonica*	SG3	41	Jazzman-2	Louisiana	*Japonica*	AD
7	FL378	International	*Japonica*	SG3	42	Jes	Louisiana	*Indica*	SG3
8	TCCP-266	International	*Indica*	SG3	43	CL162	Louisiana	*Japonica*	SG3
9	IRRI147	International	*Indica*	SG3	44	CL181	Louisiana	*Japonica*	SG7
10	Epagri	International	*Indica*	SG3	45	CL111	Louisiana	*Japonica*	SG7
11	Damodar	International	*Indica*	AD	46	CL131	Louisiana	*Japonica*	SG7
12	Chengri	International	*Indica*	SG3	47	Cypress	Louisiana	*Japonica*	SG7
13	CSR11	International	*Indica*	SG3	48	CL161	Louisiana	*Japonica*	SG7
14	PSVRC	International	*Indica*	SG3	49	LA0702085	Louisiana	*Japonica*	SG7
15	Pin Kaeo	International	*Indica*	SG7	50	CL261	Louisiana	*Japonica*	SG4
16	Dular	International	*Indica*	SG3	51	N-22	International	*Indica*	SG3
17	Moroberekan	International	*Japonica*	SG4	52	CR5272	International	*Indica*	SG3
18	Nipponbare	International	*Japonica*	AD	53	Agami	International	*Indica*	NG
19	Geumgangbyeo	International	*Indica*	SG3	54	Arang	International	*Indica*	NG
20	IR-29	International	*Indica*	NG	55	Kalia-2	International	*Indica*	SG3
21	Cocodrie	Louisiana	*Japonica*	SG7	56	SLO16	International	*Japonica*	SG3
22	R609	Louisiana	*Indica*	SG3	57	Djogolon	International	*Indica*	SG3
23	LAH10	Louisiana	*Japonica*	SG3	58	Colusa	Louisiana	*Japonica*	AD
24	LA0802140	Louisiana	*Japonica*	SG7	59	Acadia	Louisiana	*Japonica*	NG
25	Cheniere	Louisiana	*Japonica*	SG7	60	Delitus-1206	Louisiana	*Japonica*	SG5
26	Bengal	Louisiana	*Japonica*	SG4	61	Tokalon	Louisiana	*Japonica*	AD
27	CL152	Louisiana	*Japonica*	SG7	62	Evangeline	Louisiana	*Japonica*	SG5
28	Roy J	Louisiana	*Japonica*	AD	63	Pirogue	Louisiana	*Japonica*	SG4
29	Rey	Louisiana	*Japonica*	SG6	64	Rexona	Louisiana	*Japonica*	SG3
30	CL142	Louisiana	*Japonica*	SG6	65	Nira	Louisiana	*Japonica*	SG5
31	Mermentau	Louisiana	*Japonica*	SG7	66	Magnolia	Louisiana	*Japonica*	AD
32	Jupiter	Louisiana	*Japonica*	SG4	67	Lacrosse	Louisiana	*Japonica*	SG4
33	Wells	Louisiana	*Japonica*	SG6	68	Sunbonnet	Louisiana	*Japonica*	SG6
34	Catahoula	Louisiana	*Japonica*	SG7	69	Ecrevisse	Louisiana	*Japonica*	AD
35	Kalia	International	*Indica*	SG7	70	Toro	Louisiana	*Japonica*	SG6

Table A1. continued

S. N	Genotype	Source	Sub-type	Sub-group	S. N	Genotype	Source	Sub-type	Sub-group
71	Nato	Louisiana	*Japonica*	AD	106	Rexark Rogue_9262	Texas	*Japonica*	SG1
72	Saturn	Louisiana	*Japonica*	AD	107	Smooth Zenith	Texas		SG1
73	Della	Louisiana	*Japonica*	SG6	108	Short Century	Texas	*Japonica*	SG2
74	Vista	Louisiana	*Japonica*	SG4	109	Family 24	Arkansas	*Japonica*	SG1
75	Trenasse	Louisiana	*Japonica*	AD	110	Century Patna	Texas	*Japonica*	SG2
76	LA110	Louisiana	*Japonica*	SG3	111	Early Colusa	California	*Japonica*	SG8
77	Leah	Louisiana	*Japonica*	SG5	112	Rexark Rogue_9214	Texas	*Japonica*	AD
78	Toro-2	Louisiana	*Japonica*	NG	113	Century Rogue	Texas	*Japonica*	SG2
79	Mercury	Louisiana	*Japonica*	SG4	114	Nira 43	Texas	*Japonica*	AD
80	Lacassine	Louisiana	*Japonica*	AD	115	Arkose Selection	Arkansas	*Japonica*	AD
81	Jodon	Louisiana	*Japonica*	AD	116	Pecos	Texas	*Japonica*	SG1
82	Dellrose	Louisiana	*Japonica*	SG6	117	Skybonnet	Texas	*Japonica*	SG6
83	Lafitte	Louisiana	*Japonica*	SG4	118	Tebonnet	Arkansas	*Japonica*	SG6
84	Dellmati	Louisiana	*Japonica*	SG7	119	M-202	California	*Japonica*	SG8
85	Earl	Louisiana	*Japonica*	SG4	120	M-102	California	*Japonica*	SG8
86	Della-2	Louisiana	*Japonica*	SG7	121	Rico 1	Texas	*Japonica*	AD
87	Gulfrose	Texas	*Japonica*	AD	122	M-103	California	*Japonica*	SG8
88	R27	Missouri	*Japonica*	SG4	123	Katy	Arkansas	*Japonica*	SG2
89	Starbonnet	Arkansas	*Japonica*	AD	124	S-301	California	*Japonica*	SG8
90	Zenith	Arkansas	*Japonica*	AD	125	Maybelle	Texas	*Japonica*	SG1
91	Rexark	Arkansas	*Japonica*	SG3	126	Sierra	Texas	*Japonica*	SG6
92	Earlirose	California	*Japonica*	SG8	127	Lotus	Texas	*Japonica*	SG6
93	Caloro	California	*Indica*	AD	128	Neches	Texas	*Japonica*	SG6
94	Gody	California	*Japonica*	SG4	129	Carolina Gold	Texas	*japonica*	SG1
95	Bond	Arkansas	*Japonica*	AD	130	Presidio	Texas	*Japonica*	SG1
96	Newbonnet	Arkansas	*Japonica*	SG6	131	Sabine	Texas	*Japonica*	AD
97	Vegold	Arkansas	*Japonica*	SG6	132	Lavaca	Texas	*Japonica*	SG6
98	Gold Zenith	Arkansas		AD	133	MS-1995-15	Mississippi	*Japonica*	SG3
99	Belle Patna	Texas	*Japonica*	SG6	134	MS-1996-9	Mississippi	*Japonica*	SG3
100	Nova	Arkansas	*Japonica*	AD	135	Delitus	Louisiana	*Japonica*	AD
101	Palmyra	Missouri	*Japonica*	SG1	136	Salvo	Louisiana	*Japonica*	AD
102	Nova 66	Arkansas	*Japonica*	AD	137	Stormproof	Arkansas	*Japonica*	SG1
103	Glutinous Zenith	Texas	*Japonica*	SG3	138	Calady	California	*Japonica*	SG8
104	Dawn	Texas	*Japonica*	SG2	139	Zenith-2	Arkansas	*Japonica*	SG1
105	Bluebelle	Texas	*Japonica*	SG6	140	Arkansas Fortuna	Arkansas	*japonica*	SG2

Table A1. continued

S. N	Genotype	Source	Sub-type	Sub-group	S. N	Genotype	Source	Sub-type	Sub-group
141	Arkrose	Arkansas	*japonica*	SG8	174	Terso	California	Japonica	SG8
142	Prelude	Arkansas	*Japonica*	SG2	175	Texas Patna	Texas	Japonica	SG1
143	Asahi	Arkansas	*Japonica*	SG8	176	Bluebonnet	Texas	Japonica	SG2
144	Kamrose	Arkansas	*Japonica*	SG8	177	Cody	California	Japonica	SG2
145	Newrex	Texas	*Japonica*	AD	178	RD	Texas	Japonica	SG6
146	M-301	California	*Japonica*	SG8	179	Rexark-2	Arkansas	Japonica	SG1
147	S-201	California	*japonica*	SG8	180	Calrose-2	California	japonica	SG8
148	M-401	California	*Japonica*	SG8	181	TP 49	Texas	Japonica	AD
149	M-302	California	*Japonica*	SG8	182	SP 14	Texas	Japonica	AD
150	Bellemont	Texas	*Japonica*	AD	183	C-4	Texas	Japonica	SG1
151	M-201	California	Japonica	AD	184	Hill Long Grain	Texas	Japonica	AD
152	Northrose	Arkansas	Indica	AD	185	Nortai	Arkansas	Japonica	AD
153	Calrose	California	japonica	SG2	186	Brazos	Texas	Japonica	AD
154	Bluebelle-2	Texas	Japonica	SG2	187	Lebonnet	Texas	Japonica	SG2
155	Lady Wright	Arkansas	Japonica	AD	188	Saturn Rogue	Arkansas	Japonica	SG2
156	Early Prolific	Arkansas	Japonica	SG1	189	Mars	Arkansas	Japonica	AD
157	Hybrid Mix	Texas	Japonica	AD	190	Starbonnet	Arkansas	Japonica	AD
158	Hill medium	Texas	Japonica	SG3	191	Gold Nato	Arkansas		SG2
159	Glutinous Selection	Texas	Japonica	SG2	192	Earlirose-2	California	Japonica	NG
160	R-50	Missouri	Japonica	SG2	193	Early Wataribur	California	Japonica	SG8
161	MO R-500	Missouri	Japonica	AD	194	Conway	California	Japonica	NG
162	R-54	Missouri	Japonica	SG2	195	Texmont	Texas	Japonica	NG
163	R-52	Missouri	Japonica	AD	196	Alan	Arkansas	Japonica	NG
164	R-27-1	Missouri	Japonica	NG	197	Millie	Arkansas	Japonica	NG
165	Jefferson	Texas	Japonica	SG2	198	Dellmont	Texas	Japonica	NG
166	Melrose	Texas	Japonica	AD	199	Rosemont	Texas	Japonica	NG
167	Dixiebelle	Texas	Japonica	AD	200	Orion	Arkansas	Japonica	NG
168	Jasmine 85	Texas	Indica	AD	201	M-204	California	Japonica	SG8
169	Carlpearl	California	Japonica	AD	202	Adair	Arkansas	Japonica	NG
170	Madison	Texas	Japonica	SG6	203	LaGrue	Arkansas	Japonica	NG
171	Tsuri Mai	California	Japonica	SG8	204	Jackson	Texas	Japonica	NG
172	Kokubelle	California	Japonica	AD	205	Azucena	International	Indica	NG
173	Maxwell	California	Japonica	SG8					

[a] source where the variety has been developed (International means the variety has been developed in any other countries except USA), [b] *Indica* or *japonica* subtype, [c] Sub-group as classified by Structure software (SG: Subgroup, AD: Admixture, NG: Not genotyped)

Table A2. Eigen vectors and eigen values of the principal components for various agronomic traits, yield, and yield-related traits in rice genotypes under drought stress

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigen value	3.24	2.77	1.08	0.76	0.40	0.35	0.28	0.05	0.04
Variation (%)	36.05	30.81	12.04	8.49	4.46	3.97	3.17	0.54	0.47
Cumulative	36.05	66.86	78.90	87.39	91.85	95.82	98.98	99.53	100.00
DTF	0.24	0.38	0.42	0.04	0.62	0.25	-0.42	0.03	0.05
NT	-0.08	0.18	-0.80	0.44	0.25	0.18	-0.16	<0.01	0.02
LRS	-0.40	0.01	0.29	0.59	-0.17	-0.47	-0.41	-0.01	0.04
FW	0.11	0.57	<0.01	0.04	-0.27	-0.10	0.22	0.52	0.52
DW	-0.01	0.54	0.16	0.29	-0.18	0.17	0.41	-0.48	-0.36
PDMC	-0.31	-0.35	0.27	0.40	0.18	0.51	0.40	0.24	0.20
SF	0.44	-0.16	<0.01	0.28	0.44	-0.56	0.44	<0.01	-0.01
GY	0.49	-0.14	0.05	0.30	-0.34	0.19	-0.16	0.46	-0.51
HI	0.48	-0.22	0.02	0.23	-0.29	0.19	-0.16	-0.48	0.54

PC, Principal components; DTF, Days to flowering; NT, Number of tillers; LRS, Leaf rolling score; FW, Fresh plant weight (g/plant); DW, Dry plant weight (g/plant); PDMC, Plant dry matter content (%); SF, Spikelet fertility (%); GY, Grain Yield (g/plant); HI, Harvest index (%)

Table A3. Representative groups of traits identified by PCA analysis of various agronomic traits, yield, and yield-related traits in rice genotypes under drought stress

Groups	No. of variables in each group	Most representative variable	Variation within cluster (%)	Overall variation (%)
1	4	Harvest index	0.73	0.32
2	4	Fresh weight	0.68	0.30
3	1	No. of tillers	1.00	0.11

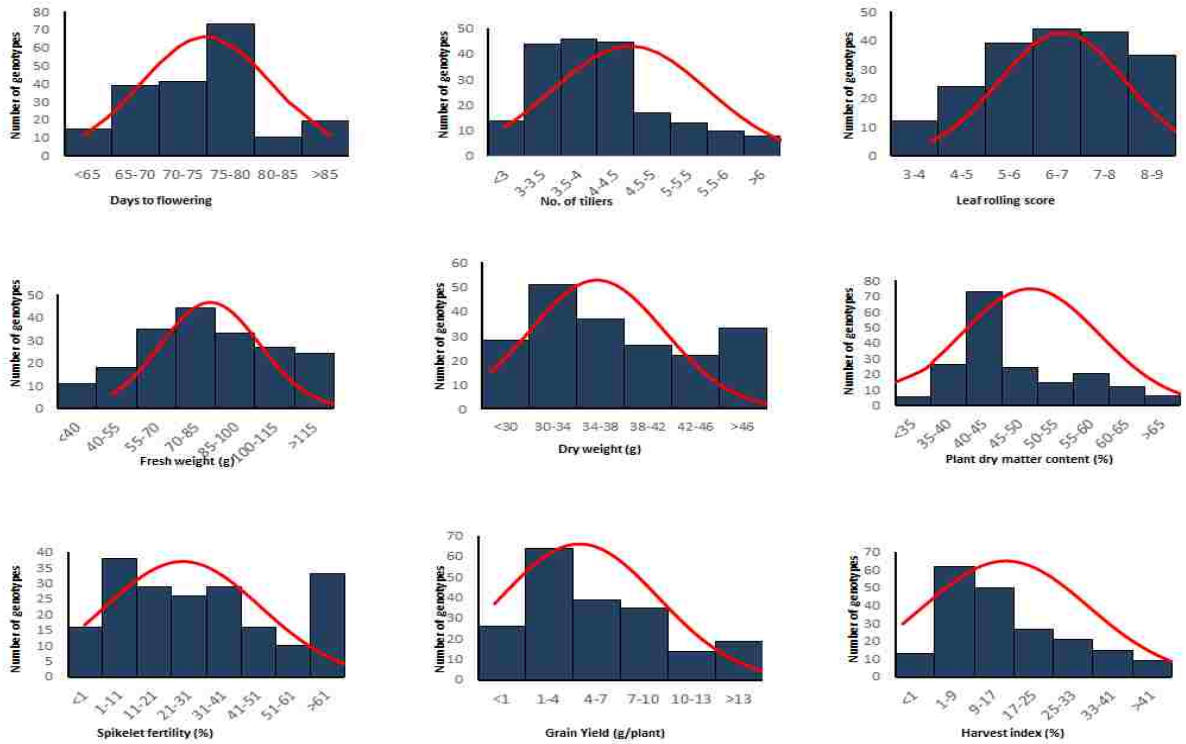

Figure A1. Frequency distribution of the various agronomic traits, yield, and yield-related traits in rice genotypes under drought stress

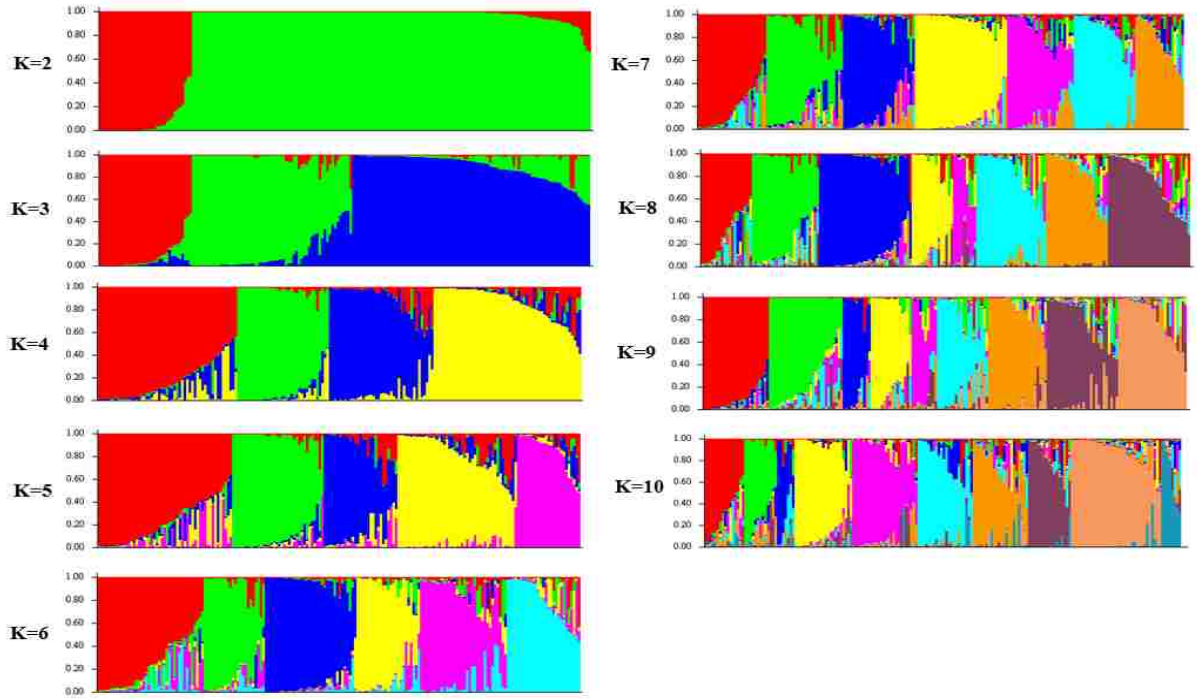

Figure A2. Estimated population structure of rice genotypes based on the membership fraction for K=2 to K=10

Appendix B. Supplementary Information for Chapter 3

Table B1. Epistatic QTLs for various root and shoot related traits identified by Interval Mapping (IM) in the Cocodrie x N-22 RIL population under water stressed condition

Phenotype	QTL1[a]	Chr. 1	Pos.1 (cM)	LeftMarker1	RightMarker1	QTL2[a]	Chr. 2	Pos. 2 (cM)	LeftMarker2	RightMarker2	LOD[b]	PVE (%)[c]	Add 1[d]	Add 2[e]	Add x Add[f]
Root Length	*qRL6.28*	6	126	S6_28019279	S6_28021750	*qRL6.07*	6	41	S6_7788675	S6_7933830	3.07	9.97	-1.26	0.83	2.62
	qRL7.03	7	20	S7_3892845	S7_3976452	*qRL7.19*	7	75	S7_19188413	S7_19306981	3.02	8.86	-0.72	1.28	-2.56
	qRL3.22	3	107	S3_22735459	S3_23020366	*qRL9.11*	9	24	S9_11218694	S9_11434669	3.37	8.67	0.50	-0.13	-2.67
	qRL8.27	8	120	S8_27458765	S8_27384353	*qRL11.07*	11	38	S11_7244556	S11_7426709	3.05	8.30	-0.94	0.36	-2.55
Shoot Length	*qSL2.32*	2	142	S2_32333805	S2_32458819	*qSL2.05*	2	2	S2_554664	S2_778122	3.62	4.35	0.31	-0.79	3.98
	qSL4.25	4	104	S4_25750452	S4_27194959	*qSL4.23*	4	75	S4_23194872	S4_23150306	3.12	4.97	3.32	-0.77	-4.07
	qSL1.40	1	215	S1_40478022	S1_40705018	*qSL4.19*	4	110	S4_19236701	S4_18975813	3.21	9.30	-3.04	2.50	-3.90
	qSL4.28	4	114	S4_28342068	S4_29179250	*qSL5.21*	5	85	S5_21788829	S5_21839740	3.42	6.69	3.16	-2.15	3.70
	qSL1.06	1	35	S1_6333407	S1_6943788	*qSL8.03*	8	25	S8_3472333	S8_3802712	3.07	3.99	-1.02	-0.21	3.72
	qSL4.02	4	14	S4_2407777	S4_4321194	*qSL10.20*	10	86	S10_20449122	S10_21032523	3.35	4.14	1.27	-1.36	3.93
	qSL6.25	6	40	S6_25081992	S6_25111550	*qSL10.18*	10	71	S10_18108623	S10_18258549	3.03	3.68	0.66	0.13	3.70
	qSL7.02	7	15	S7_2717377	S7_3316814	*qSL10.15*	10	61	S10_15894101	S10_16140874	3.25	6.60	2.98	0.39	-3.83
	qSL4.09	4	125	S4_9392529	S4_13158661	*qSL11.16*	11	53	S11_16085790	S11_16448415	3.23	3.92	0.32	0.24	-3.75
Fresh Root Mass	*qFRM1.40*	1	210	S1_40016682	S1_40478022	*qFRM1.40*	1	215	S1_40478022	S1_40705018	18.30	4.54	0.23	-0.29	-0.86
	qFRM2.19	2	77	S2_19367035	S2_20325320	*qFRM2.18*	2	72	S2_18661775	S2_19167826	7.49	3.29	-0.62	0.61	-0.56
	qFRM1.02	1	15	S1_2425271	S1_3509893	*qFRM3.04*	3	27	S3_4903639	S3_5301261	3.27	0.75	0.12	-0.04	-0.17
	qFRM1.21	1	90	S1_21230560	S1_21995746	*qFRM4.16*	4	44	S4_16795210	S4_17838166	3.59	0.82	0.17	-0.11	-0.24
	qFRM5.14	5	45	S5_14082123	S5_16517328	*qFRM5.16*	5	50	S5_16601737	S5_17246658	7.50	3.66	0.45	-0.43	-0.67
	qFRM6.04	6	26	S6_4715280	S6_5656440	*qFRM6.04*	6	21	S6_4715280	S6_5656440	13.32	3.67	0.57	-0.69	-0.55
	qFRM8.24	8	100	S8_24396362	S8_24844138	*qFRM8.23*	8	95	S8_23531495	S8_24143416	8.66	3.34	-0.61	0.64	-0.56
	qFRM5.24	5	15	S5_2536669	S5_2495045	*qFRM9.14*	9	49	S9_14554692	S9_14623034	3.21	0.53	0.03	0.01	-0.17
	qFRM11.26	11	13	S11_2666525	S11_3246051	*qFRM11.02*	11	8	S11_2188349	S11_2666525	10.71	3.86	0.40	-0.47	-0.55
	qFRM12.04	12	35	S12_4833513	S12_5581320	*qFRM12.05*	12	40	S12_5581320	S12_6566235	12.56	3.47	-0.53	0.51	-0.55

Table B1. continued

Phenotype	QTL1[a]	Chr. 1	Pos.1 (cM)	LeftMarker1	RightMarker1	QTL2[a]	Chr. 2	Pos. 2 (cM)	LeftMarker2	RightMarker2	LOD[b]	PVE (%)[c]	Add 1[d]	Add 2[e]	Add x Add[f]
Fresh Shoot Mass	*qFSM1.40*	1	210	S1_40016682	S1_40478022	*qFSM1.40*	1	215	S1_40478022	S1_40705018	10.34	9.99	1.44	-1.57	-1.80
	qFSM2.18	2	72	S2_18661775	S2_19167826	*qFSM3.36*	3	187	S3_36366411	S3_36293343	3.46	2.85	0.02	-0.16	0.52
	qFSM4.18	4	49	S4_18975813	S4_19236701	*qFSM4.15*	4	39	S4_15947149	S4_16543430	5.67	9.62	-1.73	1.49	-1.66
	qFSM5.14	5	45	S5_14082123	S5_16517328	*qFSM5.16*	5	50	S5_16601737	S5_17246658	3.15	8.93	1.72	-1.67	-1.88
	qFSM4.15	4	39	S4_15947149	S4_16543430	*qFSM6.29*	6	136	S6_29661482	S6_29794732	3.43	2.37	-0.07	0.08	0.51
	qFSM3.24	3	112	S3_24662730	S3_24845126	*qFSM11.03*	11	18	S11_3423882	S11_3485041	3.08	2.44	0.15	-0.09	-0.49
	qFSM7.08	7	50	S7_8795148	S7_9344830	*qFSM11.18*	11	3	S11_1851686	S11_1939712	3.23	2.24	-0.13	-0.04	0.50
	qFSM11.26	11	102	S11_26213113	S11_26959420	*qFSM12.18*	12	5	S12_1853909	S12_1883341	3.39	2.57	-0.05	-0.24	0.52
Number of Tillers	*qNT1.25*	1	120	S1_25275758	S1_26447134	*qNT6.15*	6	1	S6_1523304	S6_1909168	3.03	8.63	-0.01	-0.10	-0.23
	qNT2.30	2	137	S2_30823340	S2_31011182	*qNT8.22*	8	85	S8_22107509	S8_22155070	3.52	9.89	0.08	0.04	-0.21
Dry Root Mass	*qDRM6.19*	6	71	S6_19730828	S6_19918181	*qDRM6.13*	6	66	S6_13499374	S6_13177618	3.47	9.59	0.08	-0.07	-0.09
	qDRM1.21	1	90	S1_21230560	S1_21995746	*qDRM10.02*	10	11	S10_2494063	S10_2484885	3.02	9.16	0.06	0.04	0.05
	qDRM2.19	2	77	S2_19367035	S2_20325320	*qDRM11.03*	11	23	S11_3245887	S11_3855765	3.30	7.99	-0.01	0.01	0.04
	qDRM9.08	9	14	S9_8165314	S9_8942722	*qDRM12.19*	12	65	S12_19257062	S12_19492731	3.15	6.09	0.01	-0.01	-0.03
Dry Shoot Mass	*qDSM1.24*	1	110	S1_24665184	S1_24958936	*qDSM3.30*	3	152	S3_30361166	S3_30382168	3.02	4.97	-0.04	0.04	0.12
	qDSM1.09	1	60	S1_9873666	S1_10011753	*qDSM7.12*	7	55	S7_12924589	S7_13244713	3.34	5.56	0.03	-0.05	-0.11
	qDSM3.24	3	112	S3_24662730	S3_24845126	*qDSM8.15*	8	55	S8_15711098	S8_15976748	3.33	5.94	-0.01	0.06	-0.10
	qDSM1.23	1	100	S1_23171705	S1_23339178	*qDSM11.16*	11	53	S11_16085790	S11_16448415	4.59	7.10	0.05	0.15	0.17
	qDSM4.22	4	79	S4_22450799	S4_22428384	*qDSM11.07*	11	38	S11_7244556	S11_7426709	3.31	5.11	-0.03	0.02	-0.11
	qDSM11.25	11	98	S11_25112259	S11_25140979	*qDSM12.18*	12	5	S12_1853909	S12_1883341	3.12	4.71	-0.01	0.01	0.11
	qDSM2.30	2	137	S2_30823340	S2_31011182	*qDSM12.14*	12	55	S12_14722646	S12_15344105	3.29	5.40	0.03	0.02	-0.11

Table B1. continued

Phenotype	QTL1[a]	Chr. 1	Pos.1 (cM)	LeftMarker1	RightMarker1	QTL2[a]	Chr. 2	Pos. 2 (cM)	LeftMarker2	RightMarker2	LOD[b]	PVE (%)[c]	Add 1[d]	Add 2[e]	Ad d x Add[f]
Root Shoot Ratio	*qRSR1.40*	1	215	S1_40478022	S1_40705018	*qRSR1.40*	1	220	S1_40705018	S1_40884049	3.78	6.59	0.07	-0.07	-0.06
	qRSR2.19	2	77	S2_19367035	S2_20325320	*qRSR2.18*	2	72	S2_18661775	S2_19167826	3.37	6.60	-0.06	0.05	-0.06
	qRSR1.05	1	30	S1_5521992	S1_5863007	*qRSR3.24*	3	112	S3_24662730	S3_24845126	3.11	2.05	0.00	0.00	-0.02
	qRSR2.05	2	2	S2_778122	S2_554664	*qRSR3.05*	3	27	S3_4903639	S3_5301261	3.12	2.10	0.00	-0.01	-0.02
	qRSR1.07	1	45	S1_7702003	S1_8235309	*qRSR7.01*	7	5	S7_1316514	S7_1792249	3.31	2.95	0.01	-0.01	-0.02
	qRSR8.02	8	1	S8_261276	S8_498009	*qRSR10.13*	10	51	S10_13377773	S10_13599476	3.71	2.60	-0.01	-0.01	0.02
	qRSR1.21	1	90	S1_21230560	S1_21995746	*qRSR10.24*	10	11	S10_2494063	S10_2484885	3.39	2.85	0.03	0.02	0.02
	qRSR2.19	2	77	S2_19367035	S2_20325320	*qRSR11.03*	11	23	S11_3245887	S11_3855765	3.69	3.27	-0.01	0.01	0.02
	qRSR12.04	12	30	S12_4402981	S12_4833513	*qRSR12.04*	12	35	S12_4833513	S12_5581320	4.49	7.05	-0.06	0.06	-0.05
	qRSR1.23	1	100	S1_23171705	S1_23339178	*qRSR12.19*	12	65	S12_19257062	S12_19492731	3.44	2.61	0.03	0.03	0.03

[a] qRL, qSL, qFRM, qFSM, qNT, qDRM, qDSM and qRSR are QTLs for root length, shoot length, fresh root mass, fresh shoot mass, number of tillers, dry root mass, dry shoot mass and root shoot ratio respectively. The number before the decimal indicate chromosome and the number after decimal indicate the physical location of the QTL in mega base pair.
[b] logarithm of odds;
[c] Percentage of phenotypic variance explained by the QTL;
[d] Additive effect of QTL1;
[e] Additive effect of QTL2;
[f] Epistatic effect between QTL 1 and QTL 2

Table B2. Mapping of segregation distortion loci in the Cocodrie x N-22 RIL population

Chromosome	Position (cM)	Left Marker	Right Marker	Marker Interval (bp)	LOD[a]	Segregation ratio[b]	
						Cocodrie	N-22
1	95	S1_22441843	S1_22495172	53329	25.89	0.13	1
1	109	S1_24665184	S1_24958936	293752	11.39	0.31	1
1	227	S1_41353426	S1_41629534	276108	4.2	1	0.51
2	30	S2_9838728	S2_9922843	84115	3.33	0.55	1
4	51	S4_19245327	S4_19306756	61429	6.01	1	0.44
10	43	S10_11626385	S10_11951640	325255	4.35	0.5	1
10	25	S10_5314446	S10_5341825	27379	4.49	0.5	1
10	16	S10_2493909	S10_3091088	597179	5.05	0.47	1
12	0	S12_550217	S12_524851	25366	3.12	1	0.56

[a] Logarithm of odds

[b]segregation ratio is the proportion of the alleles contributed by each parent. Since we used a RIL population, the expected segregation ratio is 1:1. Segregation ratio of 0.13:1 means the ratio is skewed towards N-22 parent and does not follow the expected Mendelian ratio of 1:1

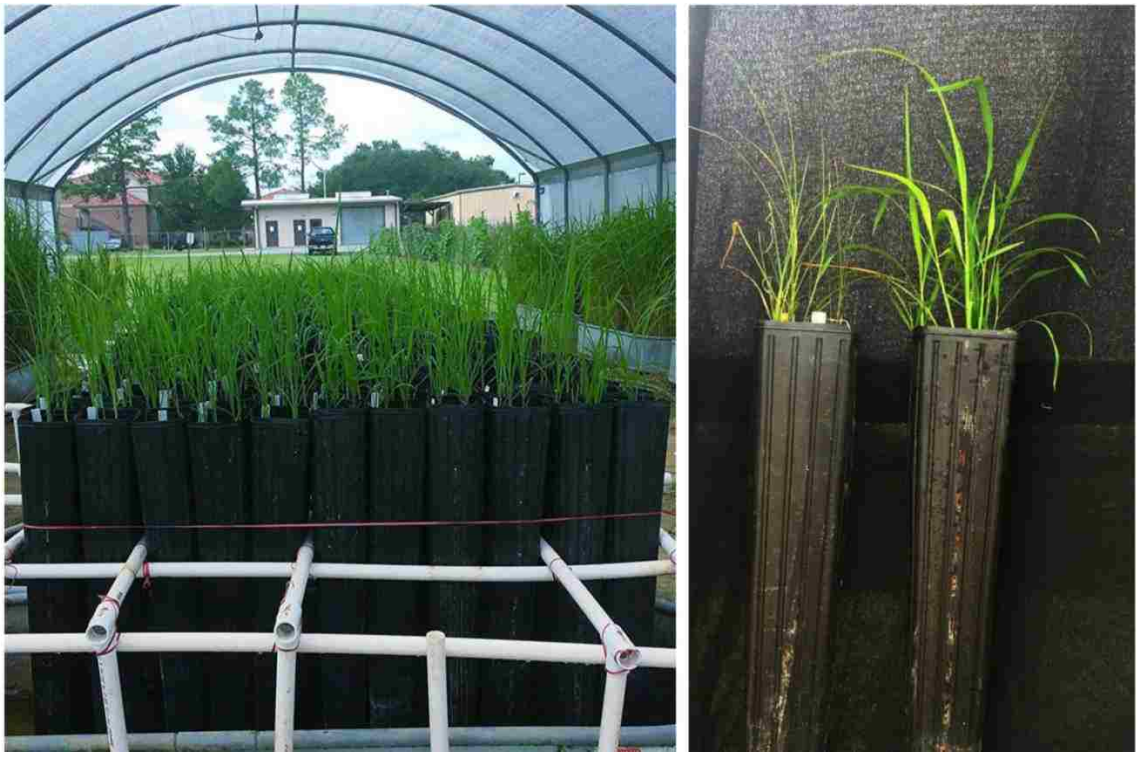

Figure B1. Experimental setup for drought experiment to evaluate root traits in rice

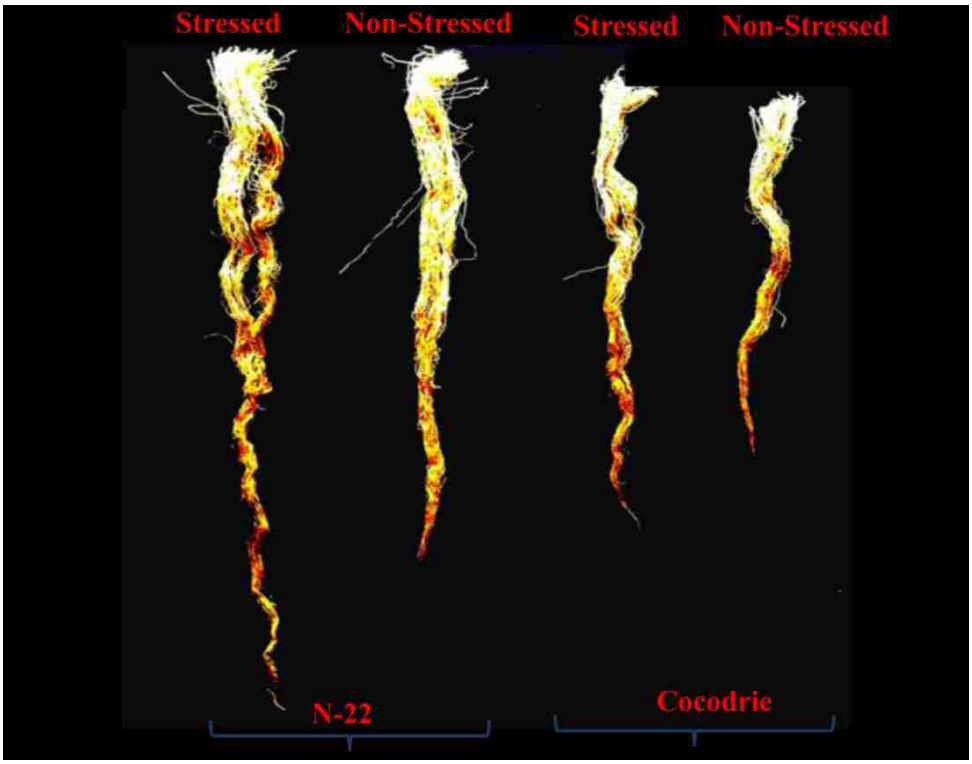

Figure B2. Difference in root length between N-22 and Cocodrie under water stress and non-stress condition

Appendix C. Supplementary Information for Chapter 4

Table C1. Analysis of variance for yield and yield-related traits in the Cocodrie × N-22 RIL population under reproductive stage drought stress.

Trait	MSS[a]	SSE[b]	F-value	P-value[c]
Days to flowering	55.54	3.49	15.87	<0.0001
Plant height (cm)	1044.45	87.85	11.89	<0.0001
Leaf rolling score	3.63	1.3	2.79	<0.0001
Dry matter content (%)	238.68	101.03	2.36	<0.0001
Spikelet fertility (%)	750.83	545.45	1.38	0.015
Grain yield under stress (g)	39.74	18.26	2.18	<0.0001
Yield index	0.11	0.04	2.22	<0.0001
Harvest index (%)	237.94	111.38	2.14	<0.0001

[a]MSS is mean sum of square
[b] SSE is sum of square of error
[c]P-value is the level of significance for the difference between the RILs

Table C2. Correlation coefficients among yield-related traits and root traits in the Cocodrie × N-22 RIL population under reproductive stage drought stress

Traits	Stress			Control		
	Root length (cm)	Dry root mass (g)	Root shoot ratio	Root length (cm)	Dry root mass (g)	Root shoot ratio
Spikelet fertility (%)	0.03	-0.08	-0.07	-0.16*	-0.13	-0.07
Grain yield under stress (g)	0.08	0.08	0.04	-0.08	-0.13	-0.09
Yield index	0.01	0.06	0.02	-0.10	-0.11	-0.03
Harvest index (%)	0.06	-0.04	0.03	-0.01	-0.13	-0.09

[*]Significant correlation between the traits at 5% level of probability

Table C3. Eigen value for the eight principle components and variance explained by each principal component

Principal component	Eigenvalue	Percent[a]	Cumulative Percent[b]
1	3.12	39.03	39.03
2	1.40	17.44	56.47
3	0.98	12.22	68.69
4	0.83	10.37	79.06
5	0.56	6.97	86.03
6	0.51	6.43	92.46
7	0.40	5.04	97.50
8	0.20	2.50	100.00

[a]Percentage of variance explained by each principal component
[b]Cumulative variance explained by each additional principal component

Table C4. Eigen vectors for each principal component for yield and yield-related traits in rice

Trait	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8
Days to flowering	-0.22	0.15	0.65	-0.67	-0.15	-0.11	0.07	-0.07
Plant height	-0.25	0.59	0.10	0.11	0.62	0.00	-0.37	0.16
Leaf rolling score	-0.33	0.43	0.11	0.36	-0.41	0.49	0.37	0.02
Plant dry matter content	-0.04	-0.48	0.66	0.47	0.27	0.11	0.00	-0.10
Spikelet fertility	0.41	-0.06	0.00	-0.35	0.30	0.76	0.08	0.11
Grain yield under stress	0.46	0.34	0.11	0.11	-0.09	0.00	-0.18	-0.77
Yield index	0.43	0.26	0.13	0.10	0.25	-0.36	0.69	0.20
Harvest index	0.45	0.12	0.28	0.15	-0.42	-0.03	-0.43	0.54

Table C5. List of additive QTLs for yield and yield-related traits in the Cocodrie × N-22 RIL population under reproductive stage drought stress using interval mapping (IM)

Trait	QTLs[a]	Chr[b]	Pos (cM)[c]	Left Marker	Right Marker	LOD[d]	PVE (%)[e]	Additive effect	Parental effect
Days to flowering	*qDTF3.01*	3	5	S3_1110340	S3_1497144	5.05	5.42	1.84	Cocodrie
	qDTF3.03	3	15	S3_3069783	S3_3408012	5.00	5.50	1.85	Cocodrie
	qDTF3.05	3	29	S3_5496857	S3_5545956	4.17	4.48	1.68	Cocodrie
	qDTF3.06	3	39	S3_6763336	S3_7002098	3.64	3.91	1.56	Cocodrie
	qDTF3.10	3	71	S3_10656190	S3_10941996	3.60	4.30	1.64	Cocodrie
	qDTF9.12	9	48	S9_12697203	S9_13964645	2.54	2.81	1.32	Cocodrie
	qDTF10.14	10	57	S10_14261588	S10_14469114	2.76	2.99	-1.39	N-22
	qDTF11.08	11	44	S11_8950871	S11_9004531	3.06	3.33	1.45	Cocodrie
Plant height	*qPH1.28*	1	138	S1_28190405	S1_28383640	2.13	2.72	-5.44	N-22
	qPH1.31	1	154	S1_31008477	S1_31033244	2.21	2.82	-5.45	N-22
	qPH1.37	1	186	S1_37273187	S1_37368297	19.65	20.13	-14.50	N-22
	qPH1.38	1	192	S1_38023681	S1_38286772	24.52	24.69	-15.99	N-22
	qPH7.02	7	17	S7_2717377	S7_3316814	3.90	5.11	7.2406	Cocodrie
Leaf rolling score	*qLRS1.37*	1	186	S1_37273187	S1_37368297	7.47	10.92	-0.56	N-22
	qLRS1.38	1	193	S1_38286772	S1_38611845	6.67	9.94	-0.53	N-22
	qLRS7.04	7	0	S7_425945	S7_553805	2.83	4.36	0.35	Cocodrie
	qLRS7.07	7	42	S7_7182433	S7_8031540	2.71	4.54	0.36	Cocodrie
	qLRS12.19	12	68	S12_19106376	S12_19379440	2.78	4.38	-0.36	N-22
Plant dry matter content	*qDM1.07*	1	43	S1_7295376	S1_7604334	2.22	5.50	2.60	Cocodrie
Spikelet fertility	*qSF1.38*	1	192	S1_38023681	S1_38286772	3.77	6.74	6.13	Cocodrie
	qSF1.39	1	203	S1_39580930	S1_39526933	2.45	4.14	4.77	Cocodrie
	qSF1.40	1	208	S1_40016682	S1_40478022	2.82	4.91	5.21	Cocodrie
	qSF3.0.6	3	0	S3_645267	S3_723901	2.89	4.82	-5.14	N-22
	qSF7.0.4	7	0	S7_425945	S7_553805	2.78	4.63	-5.07	N-22
	qSF12.24	12	98	S12_24162384	S12_24262862	2.78	4.67	-5.08	N-22
Grain yield under stress	*qGY1.42*	1	241	S1_42928362	S1_42966746	2.10	6.12	-1.02	N-22
Yield index	*qYI1.42*	1	242	S1_42981696	S1_43065133	2.24	4.99	-0.06	N-22
	qYI12.03	12	29	S12_3937337	S12_4053360	2.36	5.27	-0.06	N-22
Harvest index	*qHI1.37*	1	188	S1_37561874	S1_37740707	3.29	7.74	3.63	Cocodrie
	qHI1.42	1	241	S1_42928362	S1_42966746	3.02	7.18	-3.49	N-22
	qHI3.24	3	149	S3_24801891	S3_24951818	2.05	4.88	2.87	Cocodrie

[a]The name of the QTL. 'q' followed by the abbreviation of the trait, followed by the chromosome number, one decimal place and the physical location of the QTL in mega base pair
[b]Chromosome number
[c]Position of the QTL in genetic map
[d]Logarithm of odds value for individual QTL
[e]Phenotypic variance explained by the QTL expressed in percentage

Table C6. List of epistatic QTLs for yield and yield-related traits in the Cocodrie × N-22 RIL population under reproductive stage drought stress using inclusive composite interval mapping (ICIM)

Trait[$]	QTL 1[a]	Chr1[b]	Pos1 (cM)[c]	Left Marker1	Right Marker1	QTL 2[a]	Chr2[b]	Pos2 (cM)[c]	Left Marker 2	Right Marker 2	LOD[d]	PVE (%)[e]	Add1[f]	Add2[g]	Add x Add[h]
PH	*qPH4.16*	4	45	S4_16795210	S4_17838154	*qPH4.33*	4	135	S4_3326832	S4_33121074	4.1	9.2	1.2	2.3	-4.7
	qPH3.31	3	200	S3_31944839	S3_32058772	*qPH11.11*	11	55	S11_16931121	S11_16969972	4.8	8.1	-1.2	-0.3	-4.7
DM	*qDM3.14*	3	100	S3_14006351	S3_13604998	*qDM3.13*	3	110	S3_13604998	S3_14015710	5.0	3.2	7.1	-7.8	-6.5
SF	*qSF4.05*	4	30	S4_5728274	S4_6059987	*qSF8.06*	8	40	S8_6155282	S8_7256740	4.0	8.2	-0.2	1.0	-5.3
	qGY2.05	2	35	S2_5852996	S2_5848610	*qGY3.05*	3	30	S3_5496857	S3_5545956	4.3	9.2	0.2	-0.3	-1.4
GY	*qGY4.30*	4	125	S4_30600109	S4_31395132	*qGY8.08*	8	45	S8_8574594	S8_8874263	4.4	10.1	0.4	-0.1	-1.4
	qGY2.03	2	20	S2_3854717	S2_4310292	*qGY8.21*	8	85	S8_21174020	S8_21441966	4.9	10.8	0.3	-0.2	-1.5
YI	*qYI2.22*	2	100	S2_22663089	S2_22746914	*qYI7.39*	7	25	S7_3920664	S7_4483382	5.1	14.3	-0.1	0.1	-0.1
HI	*qHI1.08*	1	5	S1_852431	S1_1098433	*qYI4.25*	4	100	S4_25750452	S4_27194959	4.7	10.3	-1.1	0.1	4.1

[$]PH, Plant height (cm); DM, Plant dry matter content (%); SF, Spikelet fertility (%); GY, Grain yield under stress (g); YI, Yield index; HI, Harvest index (%)

[a]The name of the QTL. 'q' followed by the abbreviation of the trait, followed by the chromosome number, one decimal place and the physical location of the QTL in mega base pair

[b]Chromosome number

[c]Position of the QTL in genetic map

[d]Logarithm of odds value for the combined effect of QTL1 and QTL2

[e]Phenotypic variance explained by the combined effect of QTL1 and QTL2 expressed in percentage

[f]Additive effect of QTL1

[g]Additive effect of QTL2

[h]Epistatic effect of the interaction between the QTL1 and QTL2

Table C7. Significant gene ontology (GO) terms identified for each trait using agriGO and their classification into various sub-classes

Trait	GO accession	Term type[a]	Term	P-value
Days to flowering	GO:0080090	P	regulation of primary metabolic process	<0.01
	GO:0019222	P	regulation of metabolic process	<0.01
	GO:0031323	P	regulation of cellular metabolic process	<0.01
	GO:0050789	P	regulation of biological process	<0.01
	GO:0032774	P	RNA biosynthetic process	<0.01
	GO:0010467	P	gene expression	<0.01
	GO:0065007	P	biological regulation	<0.01
	GO:0060255	P	regulation of macromolecule metabolic process	<0.01
	GO:0016070	P	RNA metabolic process	<0.01
	GO:0051252	P	regulation of RNA metabolic process	<0.01
	GO:0006355	P	regulation of transcription, DNA-dependent	<0.01
	GO:0006351	P	transcription, DNA-dependent	<0.01
	GO:0031326	P	regulation of cellular biosynthetic process	<0.01
	GO:0045449	P	regulation of transcription	<0.01
	GO:0019219	P	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	<0.01
	GO:0010468	P	regulation of gene expression	<0.01
	GO:0009889	P	regulation of biosynthetic process	<0.01
	GO:0050794	P	regulation of cellular process	<0.01
	GO:0051171	P	regulation of nitrogen compound metabolic process	<0.01
	GO:0010556	P	regulation of macromolecule biosynthetic process	<0.01
	GO:0006350	P	transcription	0.01
	GO:0044249	P	cellular biosynthetic process	0.02
	GO:0044237	P	cellular metabolic process	0.02
	GO:0008152	P	metabolic process	0.03
	GO:0009058	P	biosynthetic process	0.03
	GO:0030528	F	transcription regulator activity	<0.01
	GO:0016491	F	oxidoreductase activity	0.04
	GO:0043226	C	organelle	0.04
	GO:0043229	C	intracellular organelle	0.04
	GO:0005622	C	intracellular	0.03

Table C7. continued

Trait	GO accession	Term type[a]	Term	P-value
Plant height	GO:0006575	P	cellular amino acid derivative metabolic process	<0.01
	GO:0006576	P	cellular biogenic amine metabolic process	<0.01
	GO:0006519	P	cellular amino acid and derivative metabolic process	<0.01
	GO:0044106	P	cellular amine metabolic process	<0.01
	GO:0009308	P	amine metabolic process	0.01
	GO:0006520	P	cellular amino acid metabolic process	0.01
	GO:0034641	P	cellular nitrogen compound metabolic process	0.02
	GO:0043565	F	sequence-specific DNA binding	0.02
	GO:0030528	F	transcription regulator activity	0.02
Leaf rolling score	GO:0008219	P	cell death	<0.01
	GO:0016265	P	death	<0.01
	GO:0012501	P	programmed cell death	<0.01
	GO:0006915	P	apoptosis	<0.01
	GO:0008234	F	cysteine-type peptidase activity	<0.01
	GO:0016757	F	transferase activity, transferring glycosyl groups	<0.01
	GO:0016758	F	transferase activity, transferring hexosyl groups	<0.01
Spikelet fertility	GO:0032774	P	RNA biosynthetic process	0.01
	GO:0045449	P	regulation of transcription	0.04
	GO:0050789	P	regulation of biological process	0.04
	GO:0006355	P	regulation of transcription, DNA-dependent	0.04
	GO:0065007	P	biological regulation	0.02
	GO:0019219	P	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	0.04
	GO:0050794	P	regulation of cellular process	0.03
	GO:0051171	P	regulation of nitrogen compound metabolic process	0.04
	GO:0051252	P	regulation of RNA metabolic process	0.04
	GO:0006350	P	transcription	0.02
	GO:0006351	P	transcription, DNA-dependent	0.01
	GO:0043169	F	cation binding	0.03
	GO:0043167	F	ion binding	0.03
	GO:0003700	F	transcription factor activity	0.03

Table C7. continued

Trait	GO accession	Term type[a]	Term	P-value
Spikelet fertility	GO:0017111	F	nucleoside-triphosphatase activity	0.04
Harvest index	GO:0070011	F	peptidase activity, acting on L-amino acid peptides	<0.01
	GO:0008233	F	peptidase activity	0.01
	GO:0004175	F	endopeptidase activity	0.03

[a]Term type: P, biological process; F, molecular function; C, cellular component

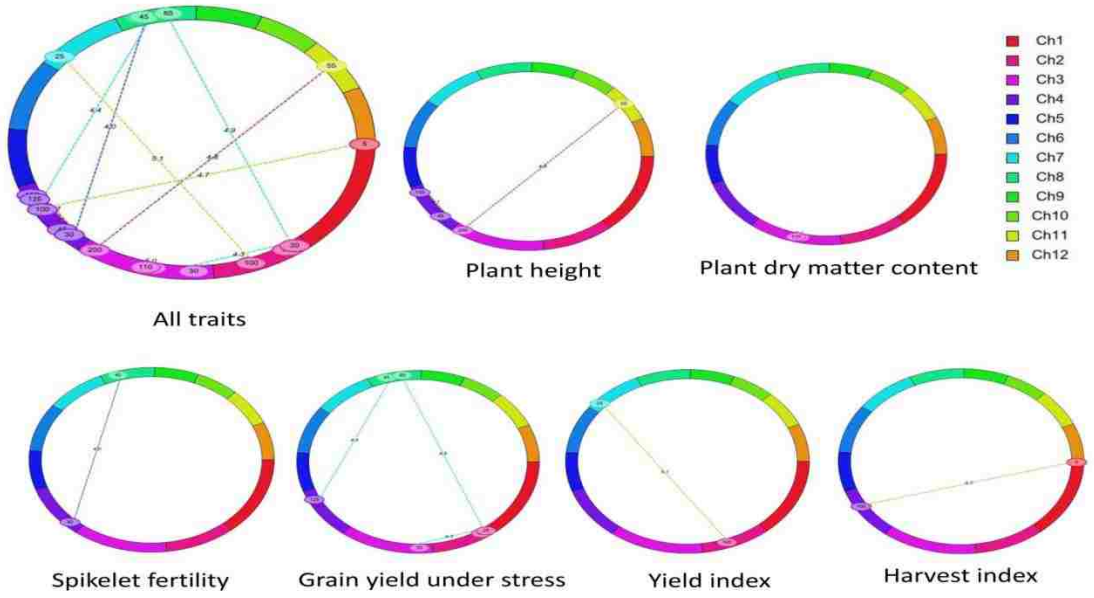

Figure C1. Genetic network of epistatic QTLs for eight yield and yield related traits under reproductive stage drought stress in rice

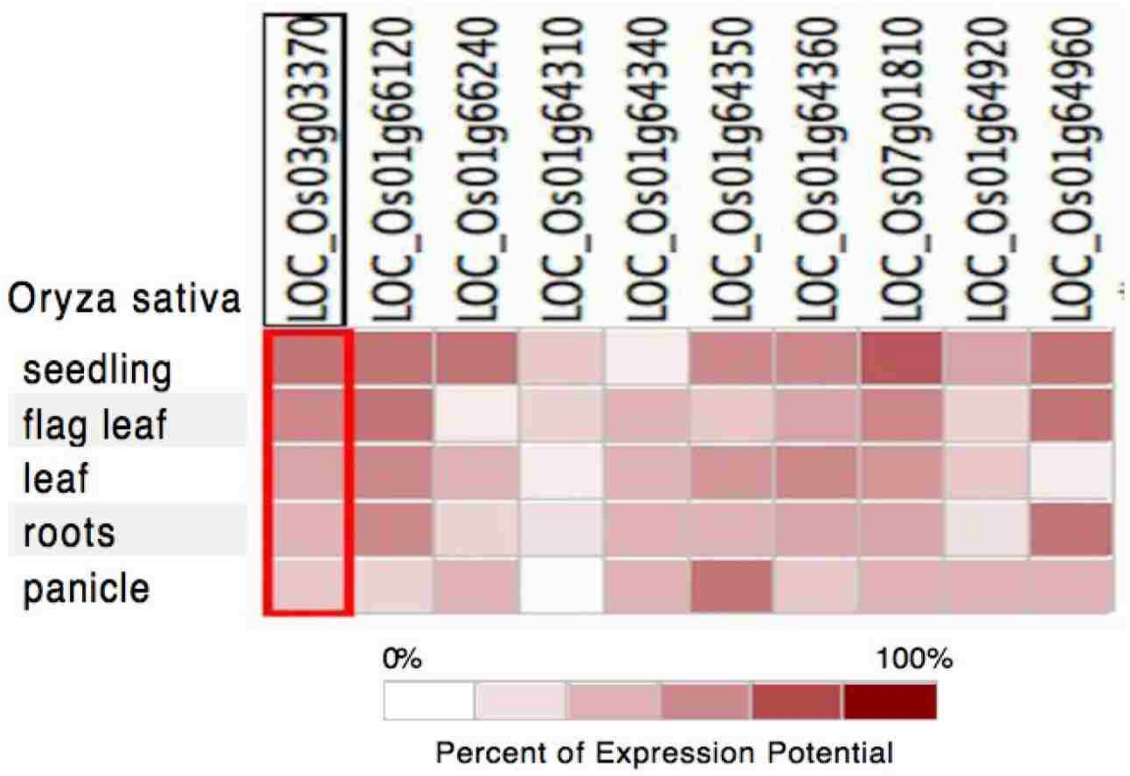

Figure C2. Expression potential of the genes in five different parts of the rice plant

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Vita

Uttam Bhattarai is a Nepalese citizen by birth. He attended his undergraduate college from Institute of Agriculture and Animal Sciences at Tribhuvan University, Nepal. After graduation, Uttam was awarded a Nepal Aid Fellowship from Indian Embassy in Nepal to pursue his master's degree in plant breeding and genetics in India. Uttam completed his master's degree from Assam Agricultural University, Jorhat India in 2013. Prior to starting his Ph.D., he did two months internship at Academia Sinica Taiwan during July and August 2013. Uttam joined Dr. Prasanta Subudhi's lab in January 2014 as a Ph.D. student. He is working on genetics of drought tolerance in USA rice genotypes. During his Ph. D studies at Louisiana state university, Uttam also graduated his master's in applied statistics as a dual degree from the same university. He has already published two first authored publications from his Ph.D. research.